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The Efficacy of Allopurinol in Suppressing Hypersensitive-like Symptoms in Wheat (Triticum aestivum L.) and in Suppressing Sunflower Rust (Puccinia helianthi) Symptoms

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BY

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This dissertation is approved as creditable and independent investigation by a candidate for the Doctor of Philosophy degree and acceptable for meeting the dissertation requirements for this degree. The acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusion of the major department.

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‘To my late mum Ethel Edith Mkandawire-nyauTegha, who sacrificed everything, supported and encouraged me to work hard in academia. My late dad, Captain Walker Gambler Mkandawire (0291), whose love and desire to succeed academically inspired me to emulate his wish’.

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LIST OF ABBREVIATIONS

ABRE	abscisic acid response elements
ACD	accelerated cell death
ANOVA	Analysis of variance
β	Beta
blm	blast lesion mimic
Bydv	barley yellow dwarf virus
cm	centimeter
CIMMYT	International Centre for Improvement Maize and Wheat
cm^2	centimeter square
CPN1	carboxypeptidase N, polypeptide 1
cpr	constitutive expresser of pathogen related
DNA	Deoxyribonucleic acid
EMS	ethylmethane sulphonate
FHB	Fusarium head blight
g	gram
HF	hydrogen fluoride
lm	lesion mimic

Ltn	leaf tip necrosis
Lr	leaf rust
Lsd	lesion stimulating disease
Mlo	Mildew Locus O
mm^2	millimeter square
MMS	methylmethane sulphonate
NAD(P)H	nicotinamide adenine dinucleotide diphosphate
NILs	Near-Isogenic Lines
NBS-LRR	Nucleotide binding site Leucine-Rich-Repeats
Pm	powdery mildew
QTL	quantitative trait loci
Rht	reduced height
RNA	Ribonucleic acid
RNAi	Ribonucleic acid interference
ROS	Reactive oxygen species
SCAR	sequence characterized amplified region
SNP	single nucleotide polymorphism
SOD	Sodium dismutase

Spl	spotted leaf
Sr	stem rust
TCA	tricarboxylic acid cycle
TDFs	transcript derived fragments
TILLING	targeted induced lesion localization IN genome
Yr	yellow rust
µM	micro molar
XA	xanthine oxidase
XDH	xanthine dehydrogenase

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ABSTRACT

THE EFFICACY OF ALLOPURINOL IN SUPPRESSING HYPERSENSITIVE -LIKE SYMPTOMS INA WHEAT (*Triticum aestivum* L.) LESION MUTANT AND IN SUPPRESSING SUNFLOWER RUST (*Puccinia helianthi*) SYMPTOMS.

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Under the supervision of Karl Glover and Bill Berzonsky

A mutant wheat (*Triticum aestivum* L.) genotype can constitutively produce hypersensitive-like flecks in the absence of any pathogen. The lesions of the mutant are thought to be the result of Reactive Oxygen Species (ROS), which are similarly implicated in hypersensitive reactions to fungal pathogens. Allopurinol can suppress ROS. Consequently, allopurinol was applied to a wheat lesion mutant to test the hypothesis that the same ROS are involved, regardless of the presence of the pathogen. 'Ning7840', a lesion mutant and 'Alsen', a related genotype that does not express the mutant phenotype were treated with different concentrations of allopurinol under controlled conditions in a greenhouse. The results reveled significant genotype × allopurinol interaction effects on grain yield, grain number, and chlorophyll content. Up to the application of 50µM allopurinol, there was a significant increase in grain yield and grain number; whereas any increase above 50µM allopurinol reduced grain yield and

grain number compared to the check treatment. Allopurinol reduced hypersensitive reaction-like symptoms in Ning7840, with the highest concentration resulting in the lowest leaf chlorosis. However, there was a decline in chlorophyll content, with allopurinol levels above 25µM applied in Ning7840. The results suggest that higher levels of allopurinol were effective in suppressing hypersensitive reaction-like symptoms in Ning7840, but the treatment impacts grain yield beyond 50µM level.

New races of sunflower rust (*Puccinia helianthi*) can overcome R-genes in sunflower (*Helianthus annuus* L.) resulting in significant yield losses. Allopurinol has effectively reduced rust-disease symptoms in other crops but has not been applied in to sunflower. Different concentrations of allopurinol were applied as a soil drench to sunflower and the concentrations of allopurinol reduced the symptoms, but genotypes did not react differently. There was a reduction in spike area, number of seeds, weight of seeds and weight of biomass with an increase in allopurinol concentrations. Allopurinol reduced rust symptoms, but this did not result in an increase in grain yield in comparison to the control.

CHAPTER 1: GENERAL INTRODUCTION

1.1. Plant-pathogen interactions

Plants and pathogens have coevolved over time, forming a "resistance-counter-resistance" relationship. This type of relationship was defined in a gene-gene model, which states that for every gene for avirulence in a pathogen there is a corresponding gene for resistance (Flor, 1942). Nevertheless, the interaction of the pathogen and host may not result in disease in the absence of a favorable environment. The interaction of three components results in disease (Grulke, 2011).

Non-host plant resistance encompasses the constitutive and inducible resistance. Inducible resistance is activated by the recognition of variant pathogen associated molecular patterns (PAMPS) by the host (Jones & Takemoto, 2004). Once activated, PAMPS set in motion a diverse group of defense systems in the host that enable the plant to resist the invading pathogen. Heath (2000) reported that inducible resistance is stable compared to the nonspecific plant resistance. The preformed barriers include thicker cell walls and surface microbial enzymes and secondary metabolites that prevent the pathogen from establishing infection structures on the host (Thordal-Christensen, 2003; Dixon, 2001; Nurnberger, et al. 2004). For instance, benzoxazinoids (Bxs) are secondary metabolites in cereals that have been implicated in defense roles (Nieymer, 1988). They are synthesized from tryptophan amino acid in the Shikimate pathway, and are very toxic, they are stored in plant vacuoles and plastids in an inactive form. They become active once the tissue is disrupted forming Bxs (Sicker, et al. 2000). Bxs have mutagenic effects on deoxyribonucleic acid (DNA), capable of reacting with amino acids in the process disrupting protein synthesis and creating phytotoxicity conditions in plants (Hashimoto &

Shudo, 1996). The Bxs confer some resistance to fusarium head blight as demonstrated in a study involving some Danish wheat cultivars. The study revealed a positive correlation between the levels of Bxs to the level of resistance against fusarium head blight (Søltoft, et al. 2008). Phytoalexins are another set of compounds that harness resistance to pathogen apart from playing a role in signaling pathways, male fertility, feeding deterrents from insect feeding, ultra violet (UV) protection and antioxidant activity (Shirley, 1998; Treutter, 2005). Some class of flavonoids play a role in harnessing resistance including the inhibition of cellulases, crosslinking of microbial enzymes, chelation of metals crucial for microbial enzyme activities and they polymerize compounds into crystals that act as physical barriers during pathogen infection process (Skadhauge, et al. 1997).

When pathogens overcome the first line of defense, the PAMPs are recognized on the host plasma membrane, activating the receptor-mediated defense system (Parker, 2003). There is a hypothesis that suggests that the acquisition of virulence factors by plants lead to some races within the pathogen to overcome resistance in plants during the evolutionary process (Abramovitch & Martin, 2004; Alfano & Collmer, 2004), as a result those plants became susceptible hosts to pathogens. Susceptible plants (PAMP-induced), does not have the ability to stop infection. They are also known as compatible hosts. Intense selection pressure on the hosts by the virulence genes lead to the coevolution of the *R* genes in the host. These *R* genes are able to recognize specific avirulence genes (*Avr*) in specific races in the pathogen hence creating specific pathogen specific cultivar resistance system (Espinosa & Alfano, 2004; Abramovitch & Martin, 2004). For instance, a study involving progeny lines derived from crosses between susceptible and Langdon(Dic-3A)10 (resistant line) with fusarium head blight pathogen, identified the

genes that are crucial in the pathogen-host recognition. The study unraveled some transcript derived fragments (TDFs) associated genes that normally have site for WRKY transcription factors and abscisic acid responsive elements (ABRE). The two factors play a vital in pathogen defensive gene regulation (Soresi, et al. 2015)

The gene-for-gene model explains race specificity and host specific resistance (*Avr/R*), as well as how the lack of gene complementarity results in susceptibility (*avr/R*) (Gabriel & Rolfe, 1990). Dawkins (1999) theorized that the genes in the pathogen and host confer extended phenotypes; implying that their effects manifest in the phenotype of another organism. The theory supports the existence of co-evolutionary interaction events between the pathogen and host (Thrall & Burdon, 2002). Several studies support co-evolutionary theory in resistant breeding in wheat. For instance, in powdery mildew (*Blumeria graminis* f. sp. *tritici* (*Bgt*)) disease management; identification of new resistant genes is an ongoing approach in containing the disease. Studies show that currently there are ~ 45 loci for resistance to powdery mildew distributed on 18 chromosomes of wheat genome (Ma, et al. 2011; Zhang, Guan, & Li, 2010). The major challenge is that most of the loci harbor qualitative genes that pathogens easily overcome (McDonald & Linde, 2002). For instance, in powdery mildew, the *Pm8* gene lost its effectiveness in the 1980s while *Pm4a* is losing its effectiveness in China. (Gao, et al. 2012) identified the *Pm46* gene that conferred resistance to all 15 races prevailing tested in the study compared to *Pm2*, which was susceptible to four races. Furthermore, the results indicated that there was no allelism between the two genes, hence confirming that the *Pm46* was a new gene.

Discovery of genes conferring resistance against pests and disease causing pathogens has been another discipline within trait improvement. Knowledge on the

pathogen's biology and life cycle is very instrumental when developing and screening lines for pathogen resistance. For instance, when breeding for fungal pathogens, there is a need for one to ascertain the type of fungi is dealing with, thus, nectrophic, biotrophic or hemibiotrophic. The knowledge of the biology of the pathogen assists in developing screening methods. Biotrophic fungi usually infect and establish a dynamic relationship with living cells from where they derive nutrients supporting their survival and multiplication. They have developed mechanisms that evade and suppress the host's defense recognition machinery, in the process enabling them to take control of the plant's cellular organization and metabolism (Gan, et al. 2012). Nectrophic pathogens, on the other hand, derive their nutrition for growth and reproduction from dead plant cells or tissues achieved by their aggressiveness and wide-ranging virulence traits they possess. They disarm host immune defense mechanisms through use of toxins or enzymes that kill the host tissues (Mengiste, 2012; Horbach, et al. 2011). The hemibiotrophic pathogens possess the traits from both the biotrophic and nectrophic pathogens. They deploy biotrophic arsenal during the initial infection process by evading the immune machinery and suppressing the cell death of the host. These events enable the fungi's hyphae to penetrate extensively in the infected tissue, which later triggers the pathogen to utilize nectrophic weaponry; thus secretion of toxins and enzymes that kill the host cells (Horbach, et al. 2011). Diseases whose causative agents are hemibitrophic, pose challenges in identifying genes that confer resistance effectively against the biotrophic and nectrophic phases found in the pathogen. Fusarium head blight (FHB) is one of the diseases caused by a pathogen with hemibiotrophic traits. Nevertheless, some quantitative trait loci (QTLs) conferring resistance to fusarium head blight have been identified and introgressed in many

elite lines of wheat. One study reported that the QTLs having genes conferring resistance against fusarium head blight belonged to glucanases, Nucleotide Binding Site Leucine-Rich-Repeats (NBS-LRR), WRKY transcription factors and UDPglycosyltransferases (Kugler, 2013). In Resistant genes against leaf rust (*Puccinia graminis*), stem rust (*Puccinia triticina*), fusarium head blight (*Fusarium graminearum*), tan spot (*Pyrenophora tritici-repentis*), wheat streak mosaic virus have been identified and introgressed into released cultivars (Lesser & Kolady, 2011). For instance, genes conferring resistance against stem rust disease include *Sr2*, *Sr5*, *Sr6*, *Sr7a*, *Sr7b*, *Sr26*, *Sr30* and *Sr36* (Knott, 1988), suggesting that there is a concerted effort to develop lines that may withstand against stem rust. Research efforts have identified some genes that confer resistance to Ug99, a virulent race. For instance, (Yu, et al. 2015), identified *Sr46*, *SrND643* (Basnet, et al. 2015) and *Sr21* (Chen, et al. 2015) are some of the resistant genes reported conferring resistance to Ug99. This illustrates the existence of co-evolution between pathogen causing stem rust and wheat lines. On the other hand, *Lr13*, *Lr14*, *Lr16*, *Lr17* and *Lr34* are some of the genes conferring resistance against leaf rust (Khan, et al. 2013). Research on yellow stripe rust fungal disease also managed to identify genes conferring resistance against the disease. Many spring and winter wheat lines possess *Yr2*, *Yr7*, *Yr9* and *Yr18* genes that confer resistance against yellow stripe rust disease (Johnson, et al. 1988; Rajaram, et al. 1988; Singh R. , 1992; Badebo, et al. 1990). Recently researchers have discovered and cloned a gene that offers protection to the leaf, stem and stripe rust and in addition, it confers resistance to powdery mildew. *Lr67* gene operates by changing the hexose transportation in infected tissues ultimately affecting the pathogen growth and establishment (Moore, et al., 2015). This breakthrough will assist in complementing the use of *Lr34* in gene

deployment and minimize the risks of the pathogens breaking the resistance mechanisms in *Lr34*. The presence of synteny between rice and wheat offers opportunity of introducing resistance genes from wheat to rice. A study reported the development of a line with *Lr34* in rice, and the transgenic lines were able to display the traits associated with *Lr34* gene such as leaf tip necrosis and production of smaller necrotic spots on leaves. The transgenic lines effectively conferred resistance to different isolates of *Magnaporthe oryzae*, a hemibiotrophic pathogen when inoculated (Krattinger, et al. 2015). Another study also showed that Lr34res was able to be introgressed into barley (*Hordeum vulgare*) and was able to confer resistance against barley leaf rust (*Puccinia hordei*) (Risk, et al. 2013). This shows that closely related species can be potential gene pools for resistant genes.

The availability of information on the interaction between hosts and pathogens at chemical, molecular and genetic level to the public domain is very crucial for resistant breeding strategy. Advancement in throughput and screening technics coupled with computation statistical power has increased the precision and reliability in gene identification, loci and QTL discovery. In the process being able to study their functions, interactions with other genes, loci and QTLs products they produce (Fiehn, 2000) and how best to improve them so that they can easily counteract the mutations occurring in pathogen's genomes that can easily hosts resistance (Clare, 1990; Jones, et al. 2007). For instance, proteomic and metabolomics approaches were used to identify proteins produced when the effectors from *Stagonospora nodorum* were introduced in a SnToxA resistant line of wheat. The study identified proteins associated with defense responses, metabolites associated with tricarboxylic acid cycle (TCA), photosynthesis and reactive oxygen species (ROS). The results from the study classified the pathogen as a nectrophic as opposed to

biotrophic (Delphine, 2012). Another study used microarray technology to study gene expression in contrasting hosts i.e. susceptible and resistant cultivars of wheat to fusarium head blight. The results showed upregulation in β-1,3-glucanase, wheatwins, and thaumatin-like proteins in resistant lines; all these pathogen related proteins. Furthermore, there was an upregulation of phenylalanine ammonia-lyase in resistant line, which plays a significant role in the activation of defense machinery against fusarium head blight pathogen (Golkari, et al. 2009). Collaborative team research involving geneticists, pathologists and plant breeders pool up enormous information, skills and expertise in identifying and validating if there is any loss in the effectiveness of resistant genes deployed in elite lines or reemergence of a virulent races like the TTKSK (Ug99 strain), the stem rust pathogen (Newcomb, 2013; Pretorius, et al. 2000). The information shows that the field of plant-pathogen has evolved and transformed over time.

1.2. Chromosome interchanging in wheat

The discovery on possibilities of chromosome interchanging and pairing between wheat and rye without challenges enabled the introduction of the 1B-1R translocation chromosome segment from rye (5R) into wheat chromosome belonging to the homeology group 1 (Shepherd, 1973; Miller & Reader, 1987; Baum & Appels, 1991). The presence of *ph*-gene in wheat enables the homologous pairing of chromosomes in wheat (Hao, et al. 2011), enabling recombination and ultimately maintaining fertility of the progeny produced. On the contrary, it prevents homoeologous chromosome pairing. Manipulation of the *ph* gene aids in the recombination between chromosomes that do not pair during meiosis, in the end increasing genetic diversity (Ji & Langridge, 1990; Wang, et al. 1977). This ensures genome stability and maximizes fertility of the wheat lines (Ji & Langridge,

1990). The discovery of *Ph1* suppressors designated as *Su1-Ph1* and *Su2-Ph1* in chromosomes 3S locus and 7S locus of *Ae. speltoides* respectively increased the homoeologous chromosome pairing in crosses involving *Triticum aestivum* and *Ae. speltoides* (Dvorak, et al. 2006). Use of molecular marker analysis has released enormous information regarding the *Ph1* locus within the genome (Sidhu, et al. 2008; Dvorak, et al. 2006; Simon, et al. 2006; Singh, et al. 2012; Wang, et al. 2002). The *ph1* gene has played a vital role in increasing diversity within the bread diversity. One of the major breakthroughs was the introduction of the 1R chromosome segments from rye into different lines of wheat (Shepherd, 1973). Lapitan,et al. (1986) developed an efficient genome staining technique that discriminated the rye from the wheat DNA sequences in a rye-wheat hybrid genome. Thus, the rye segments were brown in color while the wheat segments stained blue. The technique improved in increasing efficiency in identifying, selecting and locating positions on translocations in the rye-wheat genome crosses. The technique may assist in screening all lines that do not possess the translocations during early stages of the breeding program rather than waiting for inoculation studies. This approach may ultimately reduce costs associated with implementing greenhouse and field trials that possess a huge collection of lines that do not possess desirable genotypes (Slater, et al. 2013; Xu & Crouch, et al. 2008; Moreau, et al. 2000; Dreher, et al. 2003). The rye chromosome segment introduced in the wheat genome possessed DNA sequences that confer resistance to powdery mildew (*Erysphe graminis* DC. ex Marat f. sp. *tritici*) and stripe rust (*Puccinia striiformis* West.) (Mettin, et al. 1973; Zeller, 1973). Furthermore, studies revealed a strong linkage to genes conferring resistance against stem rust (*Puccinia graminis* Pers. f. sp. *tritici*) and leaf rust diseases (*Puccinia recondita* Rob. ex Desm. f. sp.

tritici), (Bartos & Bares, 1971; Zeller, 1973). The line Neuztuch released in Soviet Union possessed a complete 1R chromosome, while Aurora and Kavkaz possess a chromosome interchange of 1B and 1R from wheat and rye respectively (Lookhart, et al. 1996). Another set of wheat-rye chromosome interchange designated as 1AL-1RS derived from Amigo cultivar has also played a significant role in wheat cultivar development like 1BL-1RS (Zeller & Hossam, 1983). The 1AL-1RS chromosome carries genes conferring resistance against greenbugs, stripe rust, stem rust as well as powdery mildew (Zeller & Hossam, 1983). The 1BL-1RS translocation segment was introduced into CIMMYT and in many cultivars released in many wheat-producing regions like Ethiopia, USA, China, India and Pakistan (Zeller & Hossam, 1983). Studies have also revealed that the 1BL-1RS translocation chromosome segment possesses some important agronomic traits that include higher yield gains, wider adaptation potential and higher disease resistance against a wide range of diseases prevailing in different ecologies (Kim, et al. 2004). Villareal, et al. (1997) reported that the effectiveness of 1BS-1RS was only effective under moisture stress environment in wheat compared to normal moisture conditions. On the contrary, Singh, et al. (1998), Moreno-Sevilla, et al. (1995) and McKendry, et al. (1996) reported a reduction and no significant yield advantage for the presence of rye segment in the cultivars under experimentation. Some authors have hypothesized that genetic background is the culprit creating inconsistencies in yield variability in lines with rye chromosome segment in wheat (Villareal, et al. 1994; McKendry, et al. 1996; Kim, et al. 2004). Over and above, the introgression of rye segment has improved agronomic traits in wheat, which are not limited to grain yield, disease and pathogen resistance. On the contrary, there was little utilization

of the rye chromosome segment in the Australian germplasm because of negative effects of the rye chromosome segment in flower processing traits (McIntosh, et al 1995).

1.3. Durable resistance breeding against diseases in wheat

Durable resistance breeding phenomenon is a strategy that involves developing a cultivar that possess multiple genes conferring resistance against a set of pathotypes, the cultivar grown on a wider area, remaining effective for a longer period and does not possess hypersensitive inducing genes (Johnson, 1981; Bariana, et al. 2001; Johnson & Law, 1975; Mundt, 2014). Durable resistance involves the use minor genes in a line as opposed to major resistance and the objective is to slow down the disease progression. Durable resistance operates on additive effects attributed by partial resistance from minor genes (Soriano & Royo, 2015). For instance, Singh, et al. (2008) reported that 4-5 minor genes in a cultivar could adequately contain the Ug99 strain that cause stem rust of wheat even under high disease pressure environment. On the other hand, some CIMMYT lines offer moderate resistance despite possessing only 2-3 minor genes (Singh, et al. 2005). Tight linkages between the genes offers an opportunity for the genes to be easily transferred together during intermating unlike the situation where they are not tightly together they can easily break apart during meiosis (Jones, et al. 1997; Hulbert, Webb, et al. 2001; Hermann, et al. 2013). Pleotropic effect is another form through which the two genes operate in durable resistance (Polanda, et al. 2011); for instance the *ml-o* gene conferring resistance against powdery mildew in barley (Bjørnstad & Aastveit, 1990). In wheat, there is a tight linkage between *Sr2* and *Yr30*, (Singh, et al. 2000) and they use *gwm553* marker to validate the presence of this gene combination since it is tightly linked to the gene combination (Spilmeyer, et al. 2003). Another locus harbors the *Lr34/Yr18* gene complex, offers adult

plant resistance to leaf and stripe rust. Other studies confirmed that it co-segregates with other genes conferring resistance to powdery mildew (*Pm8*) and barley yellow dwarf virus (*Bydv*) and leaf tip necrosis (*Ltn*) (Spilmeyer, et al. 2005; Liang, et al. 2006). Some examples of durable resistance in wheat include resistance to stem rust in cultivar H-44 that involved the crosses between emmer (tetraploid) to bread wheat Hope (Hare & McIntosh, 1979) and Frontana and other related cultivars against leaf rust (Rajaram, et al. 1988).

Another resistant strategy that plant breeders are using in enhancing resistance is through identification of multiple QTLs that additively increases resistance against a broad spectrum of pathogens or races within the pathogen. Each QTL possesses multiple minor genes that target multiple races or pathogens hence cumulatively slowing down pathogen multiplication. This type of resistance does not provide immunity to the host, but it is durable compared to the vertical resistance. For instance, Buerstmayr, et al. (2014) identified multiple QTLs conferring resistance to leaf rust and stripe rust within the population they developed. Using this population, they may effectively contain the two diseases concurrently. Furthermore, it may take a long period before the pathogens effectively overcome resistance in the population.

1.4. Role of mutations in cultivar development

Mutation breeding involves the exposure of genetic material to mutagens resulting in the change in the DNA sequences that induce gene expression in an organism. The changes in the progeny may be in form of structural, physiological, chemical and morphological traits (Henry, et al., 2014). Mutation can be either natural or induced. Under natural mutations, natural forces induce change in the genome. For instance, when

the cell is undergoing cell division, there might be a failure in the other DNA to make a complete copy from the template. This may induce mutations on the cells arising from the DNA template that did not have sequences from the original DNA template. A second instance, arises when the DNA is undergoing repair where there repairing process may create changes in the DNA sequence than the ones in the original template. The DNA sequences if are large or were located on the section where single base mismatch creates a huge difference then they cause a mutation in the genome. Transposon elements also induce mutations when they relocate from one location to the next within the genome. The insertion of transposon may disrupt the DNA sequence that in some cases may be a functional gene sequence, in the result affecting the physiological processes in organism (Muñoz-López & García-Pérez, 2010; Lisch, 2013). Induced mutations involve the exposure of plants parts such as seeds, vegetative organs, pollen grains to mutagens, with an objective of deleting some DNA sequences with in the genome. The deletions randomly and uniformly generated within the genome, creates genetic variability that is highly required in cultivar improvement. Some of mutagens used in induced mutation include irradiation with non-ionizing agents (ultra violet rays), irradiation with ionizing agents (X-rays, gamma rays, alpha and beta rays, fast and slow neutrons), ethylmethane sulphonate (EMS), methylmethane sulphonate (MMS), hydrogen fluoride (HF), sodium azide, *N*-methyl-*N*-nitrosourea and hydroxylamine (Sikora, et al. 2011; Parry, et al. 2009). Usually the rates are lower under natural (Kondrashov & Kondrashov, 2010) than under induced mutations. Advances in molecular toolbox have incorporated mutation-breeding technologies so that they synergistic effects of the two technologies have accelerated screening of potential materials for crop improvement at early stages of cultivar

development. Use of Targeted Induced Localized Lesion IN Genome (TILLING) has gained ground in many crop breeding programmes targeting specific traits (Sikora, et al. 2011; Parry, et al. 2009; Kumar, et al. 2013; Sabetta, et al. 2011; Slade, et al. 2005; Chen, et al. 2012).

1.5. Hybrid necrosis in wheat

Plant breeder and geneticists utilize synthetics as a broader source of diversity of the abiotic, abiotic, yield and yield components traits since they serve as a bridge for transferring genes. Thus, once synthetics have been generated they are directly crossed to cultivated hexaploids, which results in normal chromosome pairing within the 3 sets of homologous chromosomes. Studies have reported using synthetic hexaploids in transferring genes from wild progenitor species resistance genes for both biotic stresses (Ma, et al. 1995; Lutz, et al. 1995) and abiotic stresses, such as salt tolerance (Gorham, et al. 1987) and cold hardness (Limin & Fowler, 1993). Quantitative traits such as yield and protein have also been transferred from wild emmer wheat *Triticum dicoccoides*, which has resulted in improved grain yields for cultivated wheat (Cantrell & Joppa, 1991). Another study revealed that *T. tauschii* increased grain yield in hexaploid lines under drought stress environments (Gororo, et al. 2002). *Ae. tauschii* has been reported to have played a vital role in being a source for genes controlling high molecular weight (HMW) glutenin subunits that are important for baking quality in wheat (Zhang, et al. 2008; Xu, et al. 2010; Wang, et al. 2012). Nevertheless, challenges have been reported with the use of synthetic hybrids in wheat breeding associated with loci complementarity in the genomes during intercrossing. Some of the challenges reported include hybrid chlorosis and hybrid necrosis, and these abnormalities are associated with mutations in the genome.

Hybrid necrosis is the progressive premature death of plant leaves and potentially the entire plant of an F_1hybrid due to the presence of *Ne1* and *Ne2* dominant genes that are located on the proximal half of chromosome 5BL and the distal half of chromosome 2BS (Chu, et al. 2006). Another study reported the presence of another gene, *NetJingY176* associated with hybrid necrosis on chromosome 2DS and it cosegregated with *XsdauK539* and *XsdauK561* markers. Furthermore, the results from the marker analysis revealed ~ 50% association between *NetJingY176* gene and the *Ae tauschii* accessions evaluated in the study suggesting that the genes is widely distributed in the lines originating from *Ae tauschii* and precautionary measures be taken when selecting parents for crosses (Xue, et al. 2015). Tsunewaki, (1992) observed a high frequency of hybrid necrosis in F_1 hybrid plants in hexaploid wheat (*Triticum aestivum* L) and tetraploid wheat (*Triticum turgidium*) genotypes. Many studies have documented the global distribution of *Ne1* and *Ne2* genes in different cultivars, and there are patterns to the geographical distribution of these genes (Tsunewaki, 1992; Pukhalaskiy, et al. 2000; Singh, et al. 2000). Hybrid necrosis usually occurs when *Ne1* and *Ne2* dominant genes are brought together in homozygous dominant alleles in an F_1 hybrid (Singh, et al. 1992; Hermsen, 1963; Hermsen, 1963). Hermsen, (1963) reported variation in hybrid necrosis, which ranged from weak (w) where there is seed production on hybrids, moderate (m), there is premature seed production on hybrids and severe (s) where there is no seed production among the hybrids. The combination of three alleles (w, m, and s) on *Ne1* and *Ne2* affect the degree of necrosis in F_1 hybrid (Singh, et al. 1992). The alleles from the genes inducing necrosis were differentiated into $Ne1^w$, $Ne1^m$, and $Ne1^s$ for *Ne1* and $Ne2^w$, $Ne2^m$ and $Ne2^s$ for *Ne2* (Zeven, 1972). Hermsen, (1963) and Zeven (1972) reported that the expressivity of hybrid necrosis is dependent on the

dosages of *Ne* alleles, genetic background and conditions where the plants are planted. These findings were further supported by (Chu, et al. 2006) who also observed that hybrid from genotypes with Ne1alleles (TA4152-60/Alsen) had moderate necrosis (F_1); but the situation was different in the F_2 generation where there was variability ranging from weak to severe necrosis attributed to segregation of the materials creating the variations in the dosages of *Ne1* and *Ne2*. The various allelic combinations and resulting hybrid necrosis can impact genetic studies and breeding objectives. For instance, a population derived from an F_1 hybrid material exhibiting (moderate necrosis) is not ideal for genetic studies since the population may not be representative of all genotypes with the loss of severely necrotic hybrids.

Hybrid chlorosis is one the growth abnormalities occurring in F_1 hybrids originating from synthetic hexaploid wheats when crossed to *Ae. tauschii* (a diploid). The incompatibility between the AB and D genomes is the major culprit creating hybrid chlorosis. The major symptom of hybrid chlorosis it that leaves turn yellowish with age despite growing in nutrient rich media and pathogen free environment (Mizuno, et al. 2010). Variability in hybrid chlorosis phenotypes has been reported in different cross combinations involving wheat related species. For instance, there was a hybrid chlorosis variability in interspecific crosses between some accessions of emmer (*Triticum. turgidium* ssp. *dicoccon* (Schrank) Thell., AABB genome) with some accessions of cultivated and wild *timopheevii* wheat (*Triticum timopheevii* Zhuk., AAGG genome) (Tsunewaki, 1970). Furthermore, this type of hybrid chlorosis in tetraploid wheat was classified as being very lethal. *Cs1* and *Cs2* genes on chromosome 5A and 4G respectively are the two complementary genes inducing hybrid chlorosis in a *Triticum turgidium* and *Triticum*

timopheevii hybrid cross (Tsunewaki, 1992). Another set of genes, *Ch1* and *Ch2* dominant complementary genes located on chromosomes 2A and 3D have been reported to induce lethal hybrid chlorosis in a tetraploid by hexaploid wheat cross (Tsunewaki, 1992; Hermsen & Waninge, 1972). Other complementary gene interactions located attributed to genes on chromosomes 2A, 3A and 2B in emmer wheat have been implicated in inducing chlorophyll related abnormalities, such as albinism, delayed virescence and striato virescence. Under albinism, the duplicate recessive genes *abn1* and *abn2* located on chromosomes 2A and 2B induce a lethal chlorosis type characterized by seedlings lacking chlorophyll from seed emergence up to the time they die since the leaves cannot photosynthesize. Plants collapse once the stored food in the seed gets finished. Sometimes, they may show some purple color in the stems and leaves due to the presence of anthocyanin compounds, which are unmasked due to lack of chlorophyll in the stems and leaves. Plants exhibiting this abnormality do not set any seeds (Tsunewaki, 2004). In delayed virescence (*dv*), the duplicate recessive genes *dv1* and *dv2* located on chromosomes 2B and 2A respectively induces the delayed virescence condition. This condition is characterized by having a normal green pigmentation (chlorophyll) in autumn but lose it during winter period. If the plants survive the winter season, they retain the chlorophyll pigmentation in spring, and set seeds though they have longer periods before they attain physiological maturity than the normal genotypes. Furthermore, the expressivity of the virescence is dependent on temperature they are exposed, studies have showed that these genotypes if raised under greenhouse conditions in winter and temperature maintained at 15^{o}C and above the plants do no develop virescence symptoms (Tsunewaki, 2004). Under striato virescence (*sv*) the genotypes have duplicates of *sv1* and

sv2 on chromosomes 3A and 2A respectively. The genotypes with these genes have normal green color in autumn and develop white colored stripes in winter, then recover during spring. The expressivity of the genes is dependent on temperature, if they are raised under controlled environments in winter at temperatures of 15^{o}C and above, the abnormalities are suppressed. These genotypes are fertile and produce viable seeds (Tsunewaki, 2004).

Many studies have revealed that hybrid necrosis and hybrid chlorosis lines have higher levels of resistance against fungal diseases. One of the reasons is that the genes conferring resistance appear to be tightly linked to the genes associated with necrosis or chlorosis. For instance, Singh, (1993) reported *Lr13* gene being tightly linked to $Ne2^{m}$ allele, which confers adult plant resistance against leaf rust. Wamishe & Millus, (2004) observed that many lines with *Lr13* possess $Ne2^{m}$, supporting the concept that there is a tight linkage between the genes. Also, genotypes with hybrid necrosis or chlorosis are observed to produce higher levels of the salicylic acid (SA), which is an important plant defense signaling compound (Alcázar, et al 2009). Nakano, et al. (2015) reported that there was upregulation of genes involved in carbohydrate metabolism and defense responses in hybrid chlorosis genotypes with mild and severe symptoms when challenged to wheat blast and powdery mildew pathogens. The hybrid necrotic lines also produce higher levels of reactive oxygen species, which have an active role in producing hypersensitive responses in plants (Mizumo, et al. 2011).

1.6. Lesion mimic lines

Lesion mimics are plants that exhibit flecks, necrotic spots or chlorosis resembling hypersensitive reaction symptoms but this occurs under pathogen free environment (Lorrain, et al., 2003). They constitutively express the hypersensitive responses because

of the mutation that occurred in their genomes. Studies have revealed the presence dominant and recessive mutations in diverse crops inducing lesion mimic phenotypes. For instance, in corn, barley, rice, wheat and Arabidopsis (Li & Bai, 2009; Yao, et al. 2009; Li, et al. 2012; Hu, et al. 1998; Penning, et al. 2004; Kang, et al., 2007; Mori, et al. 2007; Rostoks, et al. 2003; Ishikawa, 2005; Ishikawa, et al. 2003; Pilloff, et al. 2000). Advances on research in lesion mimics has lead to the discovery of loci for lesion mimics in corn (Walbot, et al. 1983) as well as in wheat (Yao, et al. 2009) and cloning of genes in some plants (Lee & McNellis, 2009; Rostoks, et al. 2006; Wang, et al. 2005; Lorrain, et al. 2004). For instance, *Spl11* and *Spl7* in rice (Yamanouchi, et al. 2002; Zeng, et al. 2004) and *Mlo* in barley (Buschges, et al. 1997) are the lesion mimic reported. Accelerated cell death (*ACD1*) (Brodersen, et al. 2002), *ACD2* (Mach, et al. 2001), carboxypeptidase N 1 (*CPN1*) (Jambunathan, et al. 2001), *HLM1* (Balague, et al. 2003), in Arabidopsis, *Les22* and *Lls1*in corn (Gray, et al, 1997; Hu, et al. 1998) are some of the lesion mimic genes reported. There is diverse phenotypic variability on lesion mimic traits such as lesion size, time when the lesions appear, color of the lesions (Johal, et al. 1995). Studies have revealed that in some mutants, lesion formation in mutants is affected by environmental cues such as temperature and light (Johal, et al. 1995).

Use of mutagens has assisted to create mutants exhibiting lesion mimics in Arabidopsis, rice, corn, barley and wheat (Li, et al. 2005; Wu, et al. 2008; Shirano, et al. 2002; Kamlofski, et al. 2007; Walbot, et al. 1983). Many studies that used the lesion mimics have assisted in providing vital information on identifying the genes involved in programmed cell death and in signaling pathways involved in disease resistance to plants (Moeder & Yoshioka, 2008). Thus, many lesion mimic mutants play significant roles in

disease resistance. In Arabidopsis, the discovery of lesion stimulating disease resistance (*lsd*), accelerated cell death (*acd*), and constitutive expresser of PR genes (*cpr5*) mutants opened a fountain of locked up genetic as well as molecular mechanisms associated with hypersensitive reaction (Weymann, et al.,1995; Greenberg, et al. 1994; Greenberg & Ausubel, 1993; Bowling, et al. 1997; Dietrich, et al. 1994). The studies with Arabidopsis mutants revealed the symptoms exhibited during pathogen-host infection and the plant is using hypersensitive mechanisms to prevent the pathogen from invading and establishing itself in the host. When the plant activates hypersensitive mechanisms, there is an increase in salicylic acid level, ethylene, jasmonic acid, callose deposition; pathogen related proteins, activation of systemic acquired resistance pathway, increased levels of reactive oxygen species, accumulation of autoflourescence compounds and sphingolipids (Weymann, et al. 1995; Dietrich, et al. 1994; Zurbriggen, et al. 2010; Mur, et al. 2008). Lesion mimic mutants in Arabidopsis revealed that the lsd and acd showed resistance to *Pseudomonas syringae* (bacteria) and *Peronospora parasitica* (oomycete) pathogens (Rate, et al. 1999; Dietrich, et al. 1994; Greenberg & Ausubel, 1993). In barley, the powdery mildew locus o (*mlo*) confers resistance to powdery mildew pathogen (*Blumeria graminis* f.sp. *hordei*) (Jarosch, et al. 1999). Rice has a couple of lesion mimic mutants reported compared to other crops. The cell death resistance (*cdr1*, *cdr2* and *cdr3*) confer resistance to rice blast (*Magnaporthe grisea*) a fungal disease (Takahashi, et al. 1999) and enhanced blast resistance (*ebr3*) confers resistance to *Xanthomonas oryzae* pv. *oryzae* (Campbell & Ronald, 2005). Yin, et al. (2000) reported that spotted leaf (*Spl8*) confers resistance to rice blast as well as bacterial blight, while blast lesion mimic (*blm*) confers resistance against all form of rice blast (Jung, et al. 2005). The *blm* gene triggers multiple

defense-associated traits, offers enhanced defenses and produces spontaneous necrotic spots. In corn, *Les9*, *Les22* and *Lls1* confer resistance against gray leaf spot (*Cercospora zea-maydis*), a fungal disease (Johal, 2007; Gray, et al. 1997; Hu, et al. 1998).

Wheat crop has lesion mimic mutants like any other crop. Kamlofski, et al. (2007), Boyd & Minchin, (2001), Boyd, et al. (2002) and Anand, et al. (2003) reported that mutants in wheat were created using transgenic approaches or use chemical mutagens. A lesion mimic's line expressing necrotic spots on the tips and edges of leaves, *Ltn1* showed resistance against leaf rust, stripe rust and powdery mildew (Krattinger, et al. 2009). Furthermore, the gene also encodes the ABC transporters other than offering resistance to pathogens. Another mutant line C591 (M8) possessed dominant genes, expressed flecks when the plants were at the booting stage, and was controlled by a single gene (monogenic) (Nair & Tomar, 2001). Another mutant line M66, expressed moderate resistance to powdery mildew and increased resistance to yellow rust and brown rust (Kinane & Jones, 2001; Boyd, et al. 2002). Li & Bai, (2009) and Yao, et al. (2009) reported the presence of a recessive gene, *lm*, located on the 1B chromosome, enhanced resistance against leaf rust. The flecks appear from the booting stage of the life cycle of the crop. The lesion mimics are reported to be low yielding compared to the non-lesion mimic lines (Yao, et al. 2009). This may negatively affect their utilization in crop improvement.

1.7. Sunflower origin, genome and utilization

Sunflower (*Helianthus annuus* L.) originated in North America (Heiser, 1955; Harter, et al. 2004) and has three closely related species namely *Helianthus annuus* ssp. *lenticularis* (wild sunflower), *Helianthus annuus* ssp. *annuus* (weed sunflower) and *Helianthus annuus* ssp. *macrocarpus* (giant sunflower). The subspecies cultivated for

edible seeds is the *H. annuus* ssp. *macrocarpus*. The *Helianthus annuus lenticularis* is highly branched, small heads, small number of rays and achenes, while the weedy sunflower is less branched relative to wild sunflower, has larger heads, produce many seeds. On the other hand, the giant sunflower is not branched, produces a single large head with huge number of seeds (Heiser, 1955). Seventeen is the basic chromosome number in the *Helianthus* genera. However, current information indicate that sunflower has 50 species and 19 subspecies an improvement on what was reported earlier (Schilling, 2006). The growth habit ranges from annual to perennial and the number of species possessing the annual and perennial growth habits estimated to 14 and 37 respectively (Schilling, 2006). However, the genus has different forms of genome, thus, diploid, tetraploid and hexaploid forms. Easiness in conducting interspecific hybridization has assisted in transferring genes controlling traits that have improved the current elite lines. Traits such as conferring resistance to pests and diseases, oil content and cytoplasmic male sterility for hybrid production have been transferred through interspecific hybridization (NDSU Extension Service, 2007). The genome size estimated to be ~ 3.5 GB and has many transposable elements, the majority being long-term repeats (Staton, et al. 2012). The presence of transposable elements in the genome creates an increased opportunity to increase changes in the genome hence increasing diversity. The transposable elements when they relocate to new places within the genome they disrupt the DNA sequences, in the process create new DNA sequences that code for new proteins that may fundamentally change the traits of the hosts (Muotri, et al. 2007; Feschotte, 2008; Slotkin & Martienssen, 2007). A study revealed that in the current hybrid populations of sunflower, there is rare genome expansion and long term repeats retrotransposons unlike in the first parental

hybridizations. The suggested hypothesis for the current scenario is that posttranscriptional activities are repressing genome expansion (Kawakami, et al. 2011). Mutation breeding approaches have also been developed and utilized in trait improvement in sunflower. For instance, a population was developed using the Target Induced Local Lesion IN Genome (TILLING) that detects natural and induced polymorphisms (SNP) for improved seed oil biosynthesis trait and other agronomic traits. Furthermore, they reported that the mutation rate was one mutation per 480kb (Kumar, et al. 2013). Another study used mutation breeding to generate population resistant to Alternaria leaf spot (*Alternaria helianthi*) in sunflower (de Oliveira, et al. 2004).

Sunflower is an industrial crop, produced globally and has multipurpose. Most commercial varieties contain approximately 39 to 50 percent of oil in the seed (NDSU Extension Service, 2007). Furthermore, the seed oil is oil used in the production of vegetable oil, while the non-seed oil used as human food and for bird food market. Traits possessed by sunflower oil such as light color, high in unsaturated fatty acids, lack of linolenic acid, bland and high smoke points make sunflower oil to fetch a premium price compared to other sources of vegetable oils. Furthermore, the presence of high oleic and linoleic fatty acids in sunflower make oil from sunflower to be highly competitive to other potential sources of vegetable oils. There is diversity in the utilization of sunflower in the industry. The cosmetic and oleochemical industries are utilizing large volumes of sunflower since the development and commercialization of sunflower cultivars high in oleic fatty acids (Luhs & Friedt, 1994). The presence fatty acid compounds in sunflower oil, other industries are using the oil to produce chemical reagents used for modifying other chemical compounds (Girardeau, et al. 2000; Leyris, et al. 2000). Products made from use

of sunflower oil as a reagent include polyester films, modified resins, plasticizers, copolymers and lacquers. Dullius, et al. (2006) reported a technology that synthesizes alkyd resins from sunflower oil used in the recycling process of polyethylene telephtalate. The pesticide industry also uses sunflower oil in their chemical formulation as surfactants and emulsifiers (Pryde & Rothfus, 1989). Sunflower oil has high oxidative stability compared to other sources of vegetable oils hence having a competitive advantage over others in the food processing industry (Kleingartner, 2002). Other studies have also revealed that sunflower oil may play a vital role in biodiesel since it has high monoalkyl esters made of long chains of fatty acids that increase the stability index in biodiesel fuels compared to other sources of vegetable oils (Moser, 2008). Sunflower oil may be used as a blend component or a substitute for other sources used in the biodiesel fuel production since it has low sulfur emissions (Moser, 2008). Commercialization of the technology will create several opportunities along the value chain in the sunflower industry.

The livestock industry uses the dehulled and non-dehulled sunflower seeds as feed for livestock such as ruminants, swine and poultry; competing with feedstocks from other sources such as corn and soybean. Meal from sunflower has high fiber content, but on the contrary, less in energy and lysine compared to meal from soybean. Protein percentage in sunflower depends on source of the seed processing; thus, dehulled seeds have higher protein percentage compared to non-dehulled or partly dehulled. For the dehulled seed the protein percent is ~ 42 while the non-dehulled the protein percent is ~28 (Rad & Keshavarz, 1976). The nonoil seed is used for by human beings, as snack as well as used in bird feeding usually is large seeded and follows different management practices (National Sunflower Association, 2016). Sunflower stalks have a potential to be utilized as silage

crop (Lardy, 2012; McGuffey & Schingoethe, 1980; Yungblut, 2003) in situations like being produced as a double crop after harvesting the first crop or where produced as an emergency crop or in situations too difficult to produce maize because drought or season being too short to produce maize.

1.8. Biotic stresses, Sunflower rust (*Puccinia helianthi*) disease

Sunflower like any other plants face many challenges during their life cycle. The biotic and abiotic stresses if not controlled affect the normal physiological, molecular and biochemical processes in the plant ultimately reducing the crop productivity or killing the plant. Further classification within biotic group results into either insect pests or diseases. Sunflower rust (*Puccinia helianthi*) is a foliar fungal disease that causes biological and economic yield losses if not controlled early in the season (Friskop, et al. 2011; NDSU Extension Service, 2007; Sendall, et al. 2006). Four other *Puccinia* species namely *Puccinia canaliculata*, *Puccinia encleliae*, *Puccinia massalis* and *Puccinia xanthii* related to *Puccinia helianthi* cause rusts diseases in wild and cultivated sunflower plants (NDSU Extension Service, 2007). *Puccinia helianthi* is a biotrophic suggesting that it requires a living host to survive (reproduce and deriving nutrients) (Kolmer, et al. 2009). The pathogen is an autoecious and macrocyclic pathogen, thus it complete its lifecycle on a single host and produces different forms of spores during its life cycle respectively (Kolmer, et al. 2009). The basidiospores are the initial spores germinating from the infected debris after overwintering or oversummering then initiate first cycle in early spring season, they infect the sunflower plant and produce the pycnia. The basidiospores arise from the telia. Usually the telia are less mobile compared to other forms of spores; nevertheless, wind can disperse them to other distant locations. The basidiospores are very

sensitive to environmental stress; hence, they require high humid and low temperature conditions. As a result, they are released during night and early morning hours (Liddell, et al. 1993). Furthermore, dispersal to long distance by wind is limited since they do not tolerate moisture stress conditions for their survival (Kolmer, et al. 2009). The pycnia produces the male (spermagonia) and female hyphae and are haploid in nature. This mechanism offers opportunities for initiating recombination ultimately creating genetic diversity in aecia recombinants. The aecia produce a second type of spores, the aeciospores, which infect plants, usually light orange in color and in clusters. Aecia production is in larger quantity, easily blown by wind to distant locations so that they effectively and efficiently infect new hosts. They penetrate new host tissues through stoma, germinate quickly, multiply fast and produce another state of spores called uredinia (urediniospores) (Friskop, et al., 2011; Markell, et al., 2009). The urediniospores are the most destructive stage of the fungi since they can cause multiple reinfections on the plant; attacking different parts of the plant including stems, leaves, bracts and petioles. Furthermore, they can be blown by wind and carried by rain splashes to other places whey they cause new infections to other hosts. As the season progresses, the urediniospores change to overwintering forms of spores to teliospores. Teliospores are dark colored, thick walled so that they resist environmental shocks such as cold and low moisture. The teliospores mark the end of the disease cycle for that particular season (Kolmer, et al. 2009; NDSU Extension Service, 2007). The cycle starts again in spring, when the telia germinate to basidia, which infects the plants and the cycle proceeds again. Temperatures of 55 to 85F coupled with high humid conditions favor the multiplication of the pathogen. The

multiplication cycle during the uredinial stage takes only 10 to 14 days to complete (Friskop, et al. 2011).

The presence of asexual and sexual reproduction in Puccinia pathogens offers opportunity for the pathogen to create new races of the pathogen through recombination (sexual) and increase the population of the new races through asexual reproduction. Sexual reproduction occurs when basidiospores produce the haploid gametes, which recombine forming aeciospores. There is multiplication of new races during the uredinial stage and teliospore stages (Kolmer, et al. 2009; Groth & Roelfs, 1982; Groth, et al. 1995; Carvalho, et al. 2011). The presence of asexual reproduction poses complex challenges in cultivar development for resistance against fungal pathogens since new strains of pathogens emerge all the time. In sunflower, studies have reported the presence of different isolates of sunflower plants (Friskop, et al. 2015) suggesting new virulent strains are emerging all the time (Kong & Kochman, 1996). Furthermore, studies also show that some strains are able to cause infection in several differential lines such as 777, 776, 737, 376, 337 and 336 isolates (Friskop, et al. 2015). Use of resistant genes is one the tools available in managing the disease. Many elite sunflower cultivars possess these resistant genes, such as R_1 (mc90), R_2+R_{10} (mc29), R_{4e} (P386), R_{4a} (HA-R1), R_5 (HA-R2), R_{4b} (HA-R3), R_{4c} (HA-R4), R_{4d} (HA-R5) (Friskop, et al., 2015) and HAR-6 (Bulos, et al. 2013). Nevertheless, use monogenic resistance in hybrids limit the lifetime of the commercial hybrids since the selection pressure for the isolate intensifies, resulting into generation of more virulent strains. Another approach involves the sequential addition of resistant genes in the already elite lines through gene pyramiding. Though the process takes a long time to add a set of R-genes using tradition breeding approaches, use of DNA based markers offers

opportunities to fast track the process. Lawson, et al. (1998) reported using sequence characterized amplified region (SCAR) a marker assisted selection procedure in identifying two genes that confer resistance against sunflower rust. Using DNA based, offers opportunities in screening the lines at an early stage of the breeding program, before inoculation treatments hence may assist in reducing the costs associated with screening large numbers of materials as happens in conventional line development (Xu & Crouch, 2008; NDSU Extension Service, 2007).

Other tools available to farming community that assists in controlling sunflower includes foliar application of with fungicides. The objective of foliar application is to limit occurrence of new infections in the process slow down disease development from forming epidemics. Timely application at appropriate dosage and the right fungicides to the plants should be the key to success pathogen control (Friskop, et al. 2011; Friskop, et al. 2015). On the hand, the fungicides if not properly managed may promote selection for virulence in the pathogen, being poisonous are hazardous to people and animals and its application is dependent on prevailing environmental conditions. For instance, the effectiveness of the fungicide may be reduced if soon after its application the location receives rainfall. If the application day or time coincides with windy or rainy weather conditions then famer cancels the application schedule. Crop rotation may assist in breaking the pathogen life cycle since the emerging spores may fail to infect the non-related crop to sunflower hence disrupting the rust incidences. Crop rotation may also reduce the development of race changes and occurrence of epidemics in sunflower growing environments (Friskop, et al. 2011; Fetch, et al. 2011). Another approach involves controlling the reservoir of the Puccinia within the location of sunflower fields. The reservoir includes the wild relatives

of sunflower plants and all the volunteer plants, since the basidiospores may kick start the life cycle in these plants and increase the multiplication of the urediniospores in these plants before they spread to sunflower plants causing epidemics.

1.9. Allopurinol chemical and xanthine oxidase

Allopurinol chemical compound with the chemical formulae [4-hydroxypyrazolo (3,4-d) pyrimidine] has multiple use in the medical fields, treating multiple ailments such as tissues and vascular associated injuries, inflammatory, gout and heart failure associated diseases (Pacher, et al. 2006; Ng, et al. 2014). Allopurinol chemical is an isomer of hypoxanthine and studies indicate that it inhibits activities of xanthine oxidase (Massey, et al. 1970; Spencer & Johns, 1970; Fujihara & Yamaguchi, 1978). Allopurinol chemical interferes with the purine synthesis pathway by disrupting the xanthine oxidoreductase, an enzyme that initiates the synthesis of purine. Xanthine oxidoreductase then oxidizes hypoxanthine to xanthine then the oxidation of xanthine ends up with uric acid. The presence of allopurinol, initiates the xanthine oxidoreductase oxidizes the allopurinol to alloxanthine binding to the reduced form of molybdenum with active sites hence inhibiting the activity of xanthine oxidase (Massey, et al.1970; Hille & Massey, 1981). Nakagawa, et al. (2007) used an RNAi method to partially and completely silence expression of xanthine dehydrogenase in Arabidopsis genotypes, where the partially and completely silenced genotypes expressed the symptoms similar to the ones expressed on allopurinol treated plants. Other studies have reported the purine decomposition is inhibited when germinating seeds, roots and suspension culture cells absorb allopurinol chemical, indicating the effect of allopurinol chemical on xanthine oxidase (Della Torre & Montalbini, 1995; Montalbini & Della Torre, 1995). Responses of the allopurinol when

the objective is to control the activity of the pathogen depend on the nature of the pathogen and genotype of the host. For instance, in tobacco when susceptible lines received a dose of allopurinol, the disease symptoms of tobacco necrosis virus and the quantity of virus titres were reduced compared to the control (Montalblin & Torre, 1996). On the contrary, when resistant tobacco cultivar received a dosage of allopurinol, there was an increase in systemic movement of virus particles of tobacco mosaic virus and distribution of necrotic spots covering the entire plant (Montalblin, 1993; Silverstri, et al. 2008). Montalbini & Della Torre, (1995) identified oxypurinol and ribonucleosides that were the metabolic products of allopurinol in plants which were responsible for preventing tobacco necrotic virus hypersensitive responses and infection induced by tobacco necrotic virus particles in allopurinol treated plants (Montalblin & Torre, 1996). In wheat, involving a compatible reaction, allopurinol treated plants showed hypersensitive responses and the intensity was proportional to the allopurinol dosage. Furthermore, pathogen growth and disease development (uredia formation and urediospore production) declined with an increase in treatment dosage. This suggests that allopurinol created non-conducive environment for pathogen establishment and spread in the susceptible host. However, in some incompatible host-pathogen interactions, treatment with allopurinol did not change the hypersensitive response, though the number of lesions were reduced (Ádám, et al. 2000). This study also reported an increase in xanthine metabolites in allopurinol treated plants, which is an indication of xanthine oxidase inhibition. In a study involving Russian Wheat Aphids on a resistant cultivar of wheat, the allopurinol-treated resistant plants recorded low levels of hydrogen peroxide, chitinase and reactive oxygen species because allopurinol chemical inhibited the activity of xanthine oxidase (Berner & Van der Westhuizen, 2010). Van Der

Westhuizen, et al. (1998a) and Van Der Westhuizen, et al. (1998b) reported that phloem feeding activities of the aphids on the resistant wheat cultivar induced the cultivar to produce the pathogen related proteins, which activates the defense related enzymes such as glucanase, chitinase and peroxidase. These are some of the traits associated with hypersensitive reaction in plants (Mur, et al. 2008).

Reactive oxygen species (ROS) plays a central role in hypersensitive responses; studies indicate that its toxicity is associated with death of tissues (Auten & Davis, 2009; Kimura, et al. 2005). ROS production has been reported in interactions involving pathogens like viruses, fungi, bacteria and insects with host plants because of induction of hypersensitive responses. Studies show that ROS is produced from enzyme NAD(P)H oxidase located in the plasmalemma or by peroxidase enzyme located in the apoplastic space and cell wall (Vanacker, et al., 1998; Bestwick, et al. 1997). Xanthine oxidase localized in the peroxisomal matrix has been implicated as the source of reactive oxygen species and is involved in the breakdown of purine compounds into nucleic acids and nucleotides that includes guanine and adenine (Zrenner, et al. 2006). Furthermore, xanthine oxidases localized in glyoxisomes are also involved in the synthesis of reactive oxygen species (Montalbini, 1995b; Sandalio, et al. 1988; Del Rio, et al. 1989; Harrison, 2002). Studies involving tobacco plants when inoculated with tobacco mosaic virus, rust inoculated studies in tobacco, wheat and beans all indicated that xanthine oxidase was the source for reactive oxygen species (Montalbini & Della Torre, 1995; Montalbini, 1992). On the other hand, under incompatible reaction in beans (Montalbini, 1992) reported that allopurinol treatment suppressed hypersensitivity responses and there was an increase in electrolyte leakage. Reactive oxygen species increases the activities of enzyme

lipoxygenase under rust infection in oats (Yamamoto & Tani, 1986) and in wheat, when inoculated with stem rust; there is an increase in lipid peroxidation (Abdou, et al. 1993).

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CHAPTER 2: EFFICACY OF ALLOPURINOL IN SUPPRESSING HYPERSENSITIVE –LIKE SYMPTOMS IN WHEAT (*Triticum aestivum* L.) LESION MUTANT

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2.1. Abstract

A mutant wheat (*Triticum aestivum* L.) genotype, 'Ning7840', is known to constitutively produce hypersensitive like flecks in the absence of pathogens. The lesions are thought to result from cell death caused by reactive oxygen species (ROS), which are similarly implicated in hypersensitive reactions to fungal pathogens. Allopurinol suppresses ROS. Consequently, allopurinol was applied to the wheat lesion mutant to test the hypothesis that the same ROS are involved, regardless of pathogen presence. Ning7840, and 'Alsen', a genotype that does not express the mutant phenotype, were treated with increasing concentrations of Allopurinol under controlled conditions in a greenhouse. Results revealed significant genotype × Allopurinol interaction effects on chlorotic area, percent chlorotic area, lesion number, chlorophyll content, and grain yield. Up to the application of 50µM Allopurinol, there was a significant increase in grain yield and number, whereas, concentrations higher than 50µM tended to reduce grain yield and number when compared with the check treatment. Allopurinol reduced hypersensitive reaction-like symptoms in Ning7840, with the highest concentration resulting in the lowest leaf chlorosis. However, allopurinol concentrations above 25µM applied to Ning7840 also resulted in decreased chlorophyll content. Results suggest that higher levels of Allopurinol were effective in suppressing hypersensitive -like symptoms in Ning7840, though grain yield is impacted beyond the 50µM level.

Key words: *Triticum aestivum* L. – lesion mutant – Allopurinol – grain yield – leaf chlorosis

2.2. Introduction

Plants that display lesion mimicry exhibit flecks, necrotic spots, or chlorosis, that resemble hypersensitive reaction symptoms in a pathogen free environment (Lorrain et al. 2003). They constitutively express hypersensitive-like responses because of the gene mutation. There are dominant and recessive mutations resulting in lesion mimic phenotypes in corn (*Zea mays*), barley (*Hordeum vulgare*), rice (*Oryza sativa*), wheat (*Triticum aestivum*) and Arabidopsis (*Arabidopsis thaliana*) (Li and Bai 2009, Yao et al. 2009, Li et al. 2012, Hu et al. 1998, Penning et al. 2004, Kang, et al. 2007, Mori et al. 2007, Rostoks et al. 2003, Ishikawa 2005 and Pillof et al. 2000). Specific loci that induce lesion mimics have been identified in corn (Walbot et al. 1983) as well as in wheat (Yao et al. 2009) and cloning of the genes has been completed in some plants (Lee and McNellis 2009, Rostoks et al 2006, Wang et al. 2005 and Lorrain et al. 2004). For instance, *Spl11* and *Spl7* in rice (Yamanouchi et al. 2002 and Zeng et al. 2004) *Mlo* in barley (Buschges et al. 1997) were successfully cloned. *ACD1* (Brodersen et al 2002), *ACD2* (Mach et al. 2001), *CPN1* (Jambunathan et al 2001), *HLM1* (Balague et al. 2003), in Arabidopsis, and *Les22* and *Ls1* in corn (Gray et al. 1997 and Hu et al. 1998) have been reported to express hypersensitive responses. There is diverse phenotypic variability on lesion mimic traits such as lesion size, time when the lesions appear, and lesion color (Johal et al. 1995). With some mutants, lesion formation is affected by environmental cues, such as temperature and light (Johal et al. 1995).

Mutagenesis has assisted in creating mutants that exhibit lesion mimics in Arabidopsis, rice, corn, barley and wheat (Li et al. 2005, Wu et al. 2008, Shirano et al. 2002, Kamlofski et al. 2007 and Walbot et al. 1983). Many studies that used the lesion

mimics have assisted in providing vital information on identifying genes involved in programmed cell death and signaling pathways involved in disease resistance (Moeder and Yoshioka 2008). Therefore, many lesion mimic mutants play significant roles in disease resistance. In Arabidopsis, the discovery of lesion stimulating disease resistance (*lsd*), accelerated cell death (*acd*), and constitutive expresser of PR genes (*cpr5*) mutants have helped to characterize hypersensitive responses (Weymann et al. 1995, Greenberg et al. 1994, Greenberg and Ausubel 1993, Bowling et al. 1997 and Dietrich et al 1994). Further, Arabidopsis mutants have revealed the symptoms exhibited during pathogen-host infection and that the plant uses hypersensitive mechanisms to prevent the pathogen from invading and establishing itself in the host. Studies have reported that there is an increase in production and release of salicylic acid, ethylene, jasmonic acid, and ROS. Furthermore, there is activation of pathogen related proteins, systemic acquired resistance pathway, accumulation of autofluorescence compounds, sphingolipids and callose deposition on infection sites. (Weymann et al. 1995, Dietrich et al. 1994, Zurbriggen et al. 2010 and Mur et al. 2008). Synergism of the activities and compounds assist in preventing the pathogen from penetrating the host tissues once hypersensitive reaction system in the host has been activated. Lesion mimics in Arabidopsis revealed that the *lsd* and *acd* genes provided resistance to *Pseudomonas syringae* (bacteria) and *Peronospora parasitica* (oomycete) pathogens (Rate et al. 1999, Dietrich et al. 1994, Greenberg and Ausubel 1993). In barley, the locus o (*mlo*) confers resistance powdery mildew (*Blumeria graminis* f.sp. *hordei*) (Jarosch et al. 1999). Rice has a couple of lesion mimic mutants reported compared to other crops. The cell death resistance genes (*cdr1*, *cdr2* and *crd3*) confer resistance to rice blast (*Magnaporthe grisea*) a fungal disease

(Takahashi et al. 1999) and enhanced blast resistance (*ebr3*) confers resistance to *Xanthomonas oryzae* pv.*oryzae* (Campbell and Ronald 2005). Yin et al. (2000), reported that spotted leaf (*Spl8*) confers resistance to rice blast as well as bacterial blight, while the blast lesion mimic gene (*blm*) confers resistance to all forms of rice blast (Jung et al. 2005). The *blm* gene triggers multiple defense-associated traits, offers enhanced defenses, and produces spontaneous necrotic spots. In corn, the *Les9*, *Les22* and *Lls1* genes confer resistance against gray leaf spot (*Cercospora zea-maydis*), a fungal disease (Johal et al. 2007, Gray et al. 1997 and Hu et al. 1998).

Like many other crops, lesion mimic mutants are known to exist in wheat. Kamlofski et al. (2007), Boyd and Minchin (2001), Boyd et al. (2002) and Anand et al. (2003) reported that wheat mutants were created using transgenic approaches or through the use of chemical mutagens. A wheat lesion mimic line that expressed necrotic spots on leaf tips and edges, *Ltn1* possessed resistance to leaf rust (*Puccini*a *triticina*), stripe rust (*Puccinia striiformis* f. sp. *tritici*) and powdery mildew (*Blumeria graminis* f. sp. *tritici*) (Krattinger et al. 2009). Furthermore, the gene also encodes the ATP-binding cassette (ABC) transporters, which are part of the complex integral protein membrane system transporting substrates; in addition to offering resistance to some pathogens. Another line C591 (M8) generated from M_3 generation of C591 seeds exposed to N-nitroso methyl urethane mutagen as reported by Nair and Tomar (2001) , was found to express flecks when plants were at the booting stage, and was found to be controlled by a single dominant gene (Nair and Tomar 2001). Another mutant line M66, expressed moderate resistance to powdery mildew and increased resistance to yellow rust and brown rust (Kinane and Jones 2001and Boyd et al. 2002). Li and Bai (2009) and Yao et

al (2009) reported the presence of a recessive gene, *lm*, located on the 1B chromosome, which conferred enhanced resistance against leaf rust. Flecks appeared during the booting stage and were found to be a product of hypersensitive reaction responses that occur in the absence of the pathogen and are constitutively expressed. Lesion mimics are generally reported to be low yielding when compared with non-lesion mimic lines (Yao et al. 2009) despite the lesion mimics possessing resistant genes against pathogens. This may negatively affect their utilization in crop improvement.

Allopurinol has the chemical formulae [4-hydroxypyrazole (3,4-d) pyrimidine] and has multiple uses in the medical field for treating multiple ailments such as tissue and vascular associated injuries, inflammatory, gout, and heart failure (Pacher et al. 2006 and Ng et al. 2014). Allopurinol is an isomer of hypoxanthine and studies indicate that it inhibits activities of xanthine oxidase (Massey et al. 1970, Spencer and Johns 1970, Fujihara and Yamaguchi 1978, Della and Montalbini 1995, Montalbini and Della 1995). Xanthine oxidase localized in the peroxisomal matrix has been implicated as the source of reactive oxygen species (ROS) and is involved in the breakdown of purine compounds into nucleic acids and nucleotides (Zrenner et al. 2006). Montalbini (1995), Sandalio et al. 1988, Del Rio et al. 1989 and Harrison (2002) reported that xanthine localized in glyoxisomes was involved in the synthesis of reactive oxygen species. Studies involving tobacco (*Nicotiana tabacum*) plants when inoculated with tobacco mosaic virus, rust inoculated studies in tobacco, wheat and beans (*Phaseolus vulgaris*) all indicate that xanthine oxidase was the source of reactive oxygen species (Montalbini and Torre 1995 and Montalbini 1992). On the other hand, under incompatible reaction in beans Montalbini (1992) reported that allopurinol suppressed hypersensitivity responses and

that there was an increase in electrolyte leakage. Allopurinol has been reported to disrupt host-pathogen interactions during the infection process. For instance, susceptible tobacco plants treated with allopurinol showed resistance to tobacco necrotic virus when compared to untreated control plants (Montalbini and Torre 1996). In what was expected to be an otherwise compatible reaction, allopurinol treated wheat plants displayed hypersensitive responses where their intensity was proportional to allopurinol dosage rates. This suggests that allopurinol either created, or helped to establish, an environment that was unconducive for pathogen establishment and spread in the susceptible host (Adam et al 2000).

'Ning7840' is a facultative Chinese hard red wheat cultivar that possesses genes conferring resistance to many fungal pathogens. Furthermore, Ning7840 constitutively produces hypersensitive flecks beginning in the booting stage because it possesses the *lm* gene (Li and Bai, 2009). On the other hand, 'Alsen' (Frohberg et al. 2006) is a publically released hard red spring wheat cultivar with an excellent level of resistance to Fusarium head blight (FHB; caused by *Fusarium graminearum* Schwabe) and is not known to produce hypersensitive flecks. The contrasting lesion mimic traits of the two cultivars enabled a study whose objective was to demonstrate that ROS and similar physiological responses to the hypersensitive responses are involved in the production of lesions in the mimic mutants.

2.5 Materials and Methods

Plant material: Ning7840, a facultative Chinese hard red spring wheat cultivar and Alsen a released hard red spring cultivar adapted to the Upper Midwest states were used in the study. Ning7840 whose pedigree is Aurora/Anhui11//Sumai3 has the *Fhb1* locus expresses a high level resistance to FHB. Furthermore, it possesses the *lm* gene and therefore constitutively produces flecks when plants are at the booting stage. Alsen has the pedigree ND674//ND2710/ND688 (Frohberg et al. 2006) and also expresses high levels of resistance to FHB. Nevertheless, it does not possess the lm gene therefore does not constitutively produce hypersensitive flecks.

A factorial randomized complete block design, replicated three times with two factors, genotype and allopurinol set at two and four levels respectively was utilized. The levels of allopurinol were 0µM (control), 25µM, 50µM and 75µM. Five seeds of each cultivar were sown in 96 7″ (height) X 6″ (top diameter) pots, and thinned to four seedlings at five days post emergence. The potting media consisted of one part loam soil and three parts Sunshine Mix-1(Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada). Incorporation of the loam soil assisted with water retention within the pots. Pots were placed on plates to collect excess drainage soon after irrigating the plants. Each treatment combination comprised of four pots within each replicate. Allopurinol treatment was first imposed five days after seedling emergence, where 250ml of each irrigation solution treatment was applied in specified pots using a soil drench method. Irrigation amounts were increased to 500ml per pot from booting stage of plant growth. Irrigation frequencies were also increased from twice to four times each week as the demand for water from the plants was higher with age. At six weeks post emergence, 5g

of N 14: P 14: K16 + minors Multicote 4 controlled release fertilizer (Haifa Nutri. Tech. Cranes Roots Blvd, Altamonte Springs, Florida, USA) was applied to each pot. Experiments were carried out during late fall to mid-winter season under greenhouse conditions where temperatures were maintained at 25 ± 3^{o}C with a 16 hour day length maintained with supplemental lighting.

Data collection: Chlorophyll content readings were collected from three leaf samples in each pot at three points (tip, mid and base) when plants were four and six weeks old. A spadmeter (KONIKA MINOLTA Inc., Osaka, Japan) was used to collect chlorophyll content in the leaves. Flag leaf samples were scanned when plants were at the grain filling stage; two leaves were randomly selected from each pot. A Canon Scan LiDE 120 (Canon Latin America, One Canon Park, Melville, NY, USA) scanner was connected to a laptop for further quantification. ASSESS 2.0 Image Analysis Software for Plant Disease Quantification (American Phytopathological Society, Pilot Knob Road, St. Paul, MN, USA) package was used to quantify the scanned photographs for total area from the sampled leaf, chlorotic area, percent chlorotic area and number of lesions.

Plants were harvested at physiological maturity, yield and yield components data were collected. Data collected included number of heads, weight of heads, above ground biomass, weight of grain, number of grains and harvest index. A seed counter (Davis Tool and Engineering Inc. Manufacturers, Montgomery, Illinois, USA) was used to count number of seeds from each pot.

Statistical analysis: Generalized linear mixed model approaches were used to analyze the data using the PROC MIXED routine in SAS (v9.4; SAS Institute Inc., Cary, NC), where number of runs and replicates were treated as random, while genotype and

Allopurinol level were treated as fixed factors. The data were tested at 0.05 alpha level to determine mean significance.

2.6 Results

2.6.1 Genotypic main effects

There were significant genotypic differences for chlorotic area, percent chlorotic area, lesion number, chlorophyll content (Table 2.1a), weight of stalks, grain weight, number of grains and harvest index (Table 2.1b) ($P < 0.05$). However, there were no genotypic differences for number of heads (Table 2.1a) and weight of heads (Table 2.1b) ($P>.05$). The chlorotic area (mm^2) was higher in Ning7840 (5,963.17 ±423.74) than in Alsen (943.64 ±423.17) (Table 2.2a). Percent chlorotic area and number of lesions were higher in Ning7840 than in Alsen (Table 2.2a). The percent chlorotic area in Ning7840 was 12.35 ± 1.05 while that of Alsen was 2.49 ± 1.05, translating into a six-fold difference. There was a four-fold difference in lesion number between Ning7840 (77 ± 2.5) and Alsen (18 ± 2.5). The chlorophyll content (SPAD value) of Ning7840 (42.49 ± 0.26) was higher than in Alsen (41.21 ± 0.26) (Table 2.2b). The weight of stalks (g) was higher in Ning7840 (44.44 ± 1.32) than in Alsen (35.82 ± 1.32) (Table 2.2c). Grain weight (g) was higher in Alsen (13.18 ± 0.39) than in Ning7840 (10.19 ± 0.39) (Table 2.2c). Number of grains was higher in Alsen (472 ± 12.09) than in Ning7840 (397 ± 12.09) (Table 2.2c, Fig. 1). Harvest index was higher in Alsen (0.255 ± 0.007) than in Ning7840 (0.139 ± 0.007) (Table 2.2c).

2.6.2 Allopurinol main effects

Allopurinol treatment main effects were observed on chlorotic area, percent chlorotic area, lesion number, chlorophyll content , number of heads (Table 2.1a), weight of heads, weight of grain and number of grains (Table 2.1b) ($P < 0.05$). There were no

significant allopurinol effects on weight of stalks and harvest index (Table 2.1b). There was a reduction in chlorotic area (mm^2) as the level of allopurinol increased from the control (7257.85 ± 599.25) to 75µM level (781.83 ± 599.25), representing a nine-fold reduction in chlorotic area. Nevertheless, there was no variation in chlorotic area between 50µM (1992.00 ± 599.25) and 75µM (781.83 ± 599.25). There was a two-fold reduction in chlorotic area from the control treatment (7257.85 ± 599.25) to the first level (25µM) of allopurinol treatment (3780.94 ± 599.25) (Table 2.2a). Higher levels of allopurinol treatment gave lower percentage values for chlorotic area compared to the control and 25µM. Thus, the percent chlorotic area for the control was 18.37 ± 1.49 (high) compared to the 25µM (6.52 ± 1.49), while the highest level (75µM) had the lowest percentage chlorotic area (1.14 ± 1.49) (Table 2.2a). There was a reduction in number of lesions with increased level of Allopurinol treatment (Table 2.2a). Thus, there was a reduction in number from 87 ± 3.56 (control) 55 ± 3.56 (25 µM), 33 ± 3.56 (50µM) to 13 ± 3.56 (75µM) (Fig. 2.12). There was a reduction in chlorophyll content (SPAD values) at higher levels of allopurinol compared to lower levels. At lower levels, there was no significant variation between the control and the 25µM level, where the chlorophyll content was 42.21 ± 0.37. At higher allopurinol levels, the chlorophyll contents were 41.86 ± 0.37 and 41.12± 0.37 for 50µM and 75µM levels, respectively (Table 2.2b). The highest allopurinol dose (75µM) had the highest number of heads (25 ± 1.2), which was not variable from the 50µM dose (24 ± 1.2) and the control (22 ± 1.2); nevertheless the number of heads in the 25µM dose was less (21 ± 1.2) than in all the doses and the control (Table 2.2b). There was variability between the 25µM and 75µM allopurinol doses on number of heads (Fig. 2.3), where the 25µM gave less (21 ± 1.2)

number of heads than the 75µM (25 ± 1.2) dose. The only variability on weight of heads (g) was between 25 µM and 75µM where the weight of heads in the former was less (17.37 ± 0.99) than in the latter (20.21 ± 0.99) (Table 2.2b). However, had no effects on weight of heads among the control (18.26 ± 0.99), 25µM (17.37 ± 0.99) and 50µM (19.92 ± 0.99) doses. Furthermore, there were no variations among 75µM (20.21 ± 0.99), 50µM (19.92 ± 0.99) and control (18.26 ± 0.99) allopurinol doses on weight of heads (Table 2.2b, Fig. 2.4).

There was variation attributed to allopurinol on grain weight between 50µM and 25µM whose weights were 12.88 ± 0.56 and 10.60 ± 0.56 respectively. There was no variation due to allopurinol treatment between the control (11.59± 0.56) and 75µM (11.66 ± 0.56) levels on grain weight (Table 2.2c). Allopurinol level 50µM gave the highest (466 ± 17.11) number of grains while the 25µM level gave the lowest (410 ± 17.11) number of grains (Table 2.2c). There was no variation in number of grains between the control and the 75µM whose number of grains were 424 ± 17.11 and 440 ± 17.11 respectively (Table 2.2c, Fig. 2.5).

2.6.3 Genotype × allopurinol interaction effects

Genotype × allopurinol treatment interaction effects were observed for chlorotic area, percent chlorotic area, lesion number, chlorophyll content (Table 2.1a) weight of stalks and grain weight (Table 2.1b) ($P < 0.05$). There were no significant genotype × allopurinol interaction effects on number of heads (Table 2.1a), weight of heads, number of grains and harvest index (Table 2.1b) ($P>.05$). The Ning7840 × control combination had the highest chlorotic area, (12,374.00 ± 847.47) followed by Ning7840 × 25µM (6,520.50 ± 847.47), then Ning7840 × 50µM (3536.03 ± 847.47) and finally Ning7840 ×

75 (1421.67 ± 847.47) (Table 2.2a). A similar trend was observed with Alsen. Alsen × control (2141.22 ± 847.47) gave the highest chlorotic area followed by Alsen × 25µM (1041.39 ± 847.47) and Alsen × 50µM (499.00 ± 847.47) while Alsen × 75µM combination gave the least (142.00 ± 847.47) chlorotic area (Table 2.2a). Application of allopurinol reduced chlorotic area by nine-fold in the Ning7840 × 75µM treatment combination compared with the Ning7840 × control combination (Fig. 2.1). On the other hand, Allopurinol treatment reduced chlorotic area by fifteen-fold with Alsen × 75µM compared to Alsen × control treatment combination. Furthermore, there were variations on chlorotic areas among Ning7840 × 50µM, Alsen × 50µM and Alsen × 25µM combinations. There was no variability on Alsen × 75µM, Alsen × 50µM and Ning7840 × 75µM treatment combinations on chlorotic area. On the contrary, there was variation between Ning7840 × control and Ning7840 × 25µM treatment combinations on chlorotic area. Furthermore, there was variability between Ning7840 × control and Ning7840 × 25µM treatment combinations compared to the rest of the treatment combinations on chlorotic area (Fig. 2.6).

There was an increase in percentage of chlorotic area associated with a reduction in allopurinol application on both genotypes. In Ning7840 × 75µM versus Ning7840 × control treatment combinations, there was a percentage increase in chlorotic area from 2.48 ± 2.10 to 30.53 ± 2.10 respectively. There was significant variation between Ning7840 × 25µM (10.54 ± 2.10) and Ning7840 × 50µM (5.84 ± 2.10) treatment combinations on percent chlorotic area. Furthermore, there was variation in percentage chlorotic area between Ning7840 × 75µM (2.48 ± 2.10) and Ning7840 × 50µM (5.84 ± 2.10). However, there was a significant between Ning7840 × control treatment

combinations with the rest of Ning7840 × allopurinol combinations. Furthermore, there variations among Ning7840 × control (30.53 ± 2.10), Ning7840 × 25µM (10.54 ± 2.10) and Ning7840 × 75µM (2.48 ± 2.10) treatment combinations on percent chlorotic area (Table 2.2a). There was a twelvefold reduction in percent chlorotic area between Ning7840 × 75µM compared to Ning7840 × control treatment combinations. A similar trend as observed in Ning7840 × allopurinol combinations on percent chlorotic area was observed in Alsen × allopurinol treatment combination on percent chlorotic area (Fig. 7). The Alsen × 75µM (0.33 ± 2.10) and Alsen × 50µM (0.97 ± 2.10) combinations gave the lower percent chlorotic area compared to the Alsen × control (6.18 ± 2.10) treatment combination. There were significant variations on percent chlorotic amongst the Alsen × allopurinol treatment combinations. On the contrary, there was no variation between Ning7840 × 50µM (5.84 ± 2.10) and Alsen × control (6.18 ± 2.10) treatment combinations on percent chlorotic area. There was a nineteenfold reduction in percent chlorotic area through the application of higher level of allopurinol on Alsen compared to the control (Fig. 2.7).

There was a reduction in number of lesions with an increase in level of allopurinol chemical in both genotypes (Fig. 2.12). Thus, there was a sixfold reduction in number of lesions from both the Alsen × 75µM and Ning7840 × µM to Alsen × control and Ning7840 × control treatments combinations respectively. The Ning7840 × control combination had the highest number of lesions (133 ± 5.03), followed by Ning7840 × 25µM (94 ± 5.03), Ning7840 × 50µM (56 ± 5.03) while the Ning7840 × 75µM had the lowest (23 ± 5.03) number of lesions with Ning7840 as reference genotype (Table 2.2a). Furthermore, there was high variability with the Ning7840 × allopurinol treatment

combinations on number of lesions. Alsen × 75µM gave the lowest number of lesions (8 ± 5.03) compared to Alsen × 50µM (10 ± 5.03), Alsen × 25µM (16 ± 5.03) and Alsen × control (44 ± 5.03). There was no variability on number of lesions between Alsen × 50µM and Alsen × 25µM and insignificant variation to Alsen × 75µM; however, there was significant variation between Alsen × control and the rest of the Alsen × allopurinol treatment combinations (Table 2.2a, Fig. 2.8, Fig. 2.12).

The chlorophyll content was higher in Ning7840 × control (43.71 ± 0.53), followed by Ning7840 × 25µM (43.28 ± 0.53), Ning7840 × 50µM (42.91 ± 0.53) while Ning7840 × 75µM gave the least (40.00 ± 0.53) chlorophyll content, when Ning7840 is a genotype. Thus, higher dosages of allopurinol negatively reduced the amount of chlorophyll in the leaves of Ning7840. There was no variability in chlorophyll content between Ning7840 × control and Ning7840 × 25µM, but they were different from Ning7840 × 50µM and Ning7840 × 75µM (Fig. 2.4). In Alsen × allopurinol treatment, the scenario was different from the Ning7840 × allopurinol treatment on chlorophyll content. Higher dosages of allopurinol × Alsen gave higher chlorophyll content unlike lower dosages of allopurinol × Alsen combinations. Thus, the Alsen × 75µM (42.18 ± 0.53) and Alsen × 50µM (41.51 ± 0.53) gave higher chlorophyll content than Alsen × 25µM (40.71 ± 0.53) and Alsen × control (40.50 ± 0.53) treatment combination. There was a significantly variation on chlorophyll content between Alsen × 75µM treatment combination against other Alsen × allopurinol combination. There was no variation between Alsen × control (40.71 ± 0.53), Alsen × 25µM (40.45 ± 0.53) and Alsen × 50µM (41.51 ± 0.53) treatment combinations. Furthermore, there was no variation among Ning7840 × 75µM (40.01 ± 0.53), Alsen × control (40.71 ± 0.53), Alsen × 25µM (40.45

± 0.53) and Alsen × 50µM (41.51 ± 0.53) treatment combinations. There was no variability attributed to genotype × allopurinol interaction on Ning7840 × control (43.71 ± 0.53), Ning7840 × 25µM (43.28 ± 0.53), Ning7840 × 50µM (42.91 ± 0.53), and Alsen × 75µM (42.81 ± 0.53) treatment combinations on chlorophyll content (Table 2.2b) (Fig 2.9).

There was a significant genotype × allopurinol treatment interaction on weight of stalks (Table 2.1b). The range in weight of stalks (g) ranged from 32.88 ± 2.65 to 48.43 ± 2.65 in Alsen × 25µM and Ning7840 × 25µM treatment combinations respectively (Table 2.2c). There was no variability on weight of stalks across the allopurinol level when Alsen was a genotype (Fig. 10). When Ning7840 was a genotype, the only variability was between 25µM and control. However, there were no variations on weight of stalks in plants grown in allopurinol media. Furthermore, there was no variation between plants grown in the control media versus the plants grown in 50µM and 75µM allopurinol doses media (Fig. 2.10).

There was variability attributed to genotype × allopurinol interaction on grain weight (Table 2.1b). The range in grain weight ranged from 9.15 ± 0.79 to 15.05 ± 0.79 in Ning7840 × 75µM and Alsen × 50µM treatment combinations respectively (Table 2.2c). There was no variability in grain weight where Ning7840 was used as a genotype across all the allopurinol levels. On the contrary, where Alsen was a genotype significant responses on grain weight were observed across different levels of Allopurinol (Fig 2.5). The grain weight ranged from 10.95 ± 0.79 to 15.05 ± 0.79 from Alsen × 25µM and Alsen × 50µM respectively. The grain weight from Alsen × 50µM (15.05 ± 0.79) was significantly differently from Alsen × control (12.56 ± 0.79) and Alsen × 25µM (10.95 ±

0.79) but not significantly different from Alsen × 75µM (14.17 ± 0.79). There was variability in grain weight from Alsen × 50µM (15.05 ± 0.79) and the rest of the genotype × allopurinol treatment combinations with an exception of Alsen × 75µM (14.17 ± 0.79). There was no grain weight variability among Ning7840 × control, Ning7840 × 25µM, Ning7840 × 50µM Alsen × control and Alsen × 25µM (Fig 2.11).

2.6.4 Correlations between traits

There were very strong significant positive correlations on weight of grain and number of grains (0.9717), weight of heads and weight of grain (0.9609), weight of heads and number of grain (0.9398), number of heads and weight of heads (0.9304), weight of heads and weight of stalks (0.8908) and number of heads and number of grain (0.8869) (*P<.05*). Furthermore, some very strong significant positive correlations were observed on number of grain and weight of grain (0.8786), chlorotic area and percent chlorotic area (0.8727**),** weight of stalks and weight of grain (0.8482), number of grain and weight of stalks (0.8364), number of heads and weight of stalks (0.8279), chlorotic area and number of lesions (0.7729) and percent chlorotic area and number of lesions (0.6893) (*P<.05*). Weak but positive correlations were observed on weight of grain and harvest index (0.3092), number of lesion and chlorophyll content (0.3009), number of grains and harvest index (0.2988), number of heads and harvest index (0.2399), harvest index and chlorotic area (0.2381) and chlorotic area and chlorophyll content (0.2222) (*P<.05*). Furthermore, very weak but significant correlations were observed on percent chlorotic area and chlorophyll (0.2104), weight of stalks and lesion number (0.1955), total leaf area and lesion number (0.1906) and total leaf area and chlorotic area (0.1835) (*P<.05*) (Table 2.3).

Negative significant correlations were observed on harvest index and lesion number (-0.3292), harvest index and total leaf area (-0.2052), weight of grain and chlorophyll content (-0.1963) and number of grain and chlorophyll content (-0.1879) ($P<.05$). Furthermore negative weak correlations were observed on weight of stalks and chlorophyll (-0.1747), harvest index and percent chlorotic area (-0.1694), weight of heads and chlorophyll content (-0.1668) and harvest index and chlorophyll content (-0.1623) ($P<.05$) (Table 2.3). There were no correlations on harvest index and weight of stalks, total leaf area and weight of stalks, chlorotic area and weight of stalks, percent chlorotic area and weight of stalks, number of heads and total leaf area, number of heads and chlorotic area, number of heads and percent chlorotic area ($P>0.05$) (Table 2.3). No significant correlation were observed on number of heads and lesion number, number of heads and chlorophyll content, weight of heads and total leaf area, weight of heads and chlorotic area, weight of heads and percent chlorotic area, weight of heads and number of lesions, weight of grain and total leaf area, weight of grain and chlorotic area ($P>0.05$). Furthermore, there were no significant correlations on weight of grain and percent chlorotic area, weight of grain and lesion number, number of grain and total leaf area, number of grain and percent chlorotic area, number of grains and lesion number, total leaf area and percent chlorotic area and total leaf area and chlorophyll content ($P>0.05$) (Table 2.3).

2.7 Discussion

There were allopurinol, genotype and genotype × allopurinol interaction effects on chlorotic area. The chlorotic area in Alsen (943.64 ± 423.74) was less than in Ning7840 (5963.17 ± 423.74), the huge variation is attributed to the genotypic differences in the lines. Ning7840 is a lesion mimic line that produces lesion flecks as opposed to Alsen line, a commercial cultivar. The sixfold genotypic difference creates the opportunity for executing effective allopurinol studies on the lines, otherwise would pose challenges in differentiating phenotypic differences. Allopurinol was effective in reducing the chlorotic area evidenced by a reduction in chlorotic area with an increase in the dosage of allopurinol treatment. Higher level of allopurinol treatments (75µM) had the least (781.83 ± 599.25) chlorotic area as opposed to the control (7257.85 ± 599.25) and the 25µM (3780.94 ± 599.25) treatments. The reduction in chlorotic area between control and the 25µM was approximately twofold; a similar margin was observed between 25µM and 50µM allopurinol levels. An approximately threefold reduction in chlorotic area was observed between 50µM and 75µM allopurinol levels. A ninefold reduction between 75µM and the control allopurinol treatments indicate the effectiveness of the higher dosage in suppressing mechanisms that influence chlorotic area formation and growth. Allopurinol is a chemical that suppresses the xanthine oxidase activity and subsequently reducing the production of reactive oxygen species in the presence of superoxide dismutase (Montalbini, 1992). In the case of Ning7840, the line constitutively produces lesions and flecks resembling hypersensitive reaction spots on leaves during booting stage against leaf rust (Li and Bai, 2009). The results from the study show that there was a huge variability on chlorotic area among different allopurinol

treatments, with treatments with lower (control and 25µM) allopurinol dosage having larger chlorotic area unlike the treatments with higher dosages (50µM and 75µM) of allopurinol. The reduction in chlorotic area with higher rates of allopurinol suggests that the chemical was able to reduce the activity of xanthine oxidase that is crucial for oxygen reaction species. Oxygen reaction species plays a vital role in hypersensitive reaction process that results in killing of cells in places where the pathogen tries to get entry and established in a host since they are toxic (Harrison, 2002). The inactivation of xanthine oxidase in allopurinol treated plants resulted into the reduction of chlorotic area, suggesting that allopurinol was effective in reducing the chlorotic area. Montalbini and Torre (1996) reported that tobacco plants treated with allopurinol displayed less necrotic symptoms compared to untreated plants when inoculated with necrotic virus. The results in the study agree with the observations reported by Montalbini and Torre (1996) study despite that in the former study plants were not inoculated with a pathogen, but the mechanism was similar. Another study also reported that there was a delay in the onset of flecks, reduction in pustule sizes and density on wheat and bean plants under allopurinol treatment compared to plants under zero allopurinol treatment days after getting inoculation treatment (Marte and Montalbini, 1999). The study also showed that higher dosage of allopurinol (60µM) on plants resulted into the production of very tiny sori from the pathogens and increased the number of days before the onset of flecks on the host (Marte and Montalbini, 1999). The symptoms are similar to the observations made in the study, since higher levels of allopurinol resulted into reduced chlorotic area. The results also suggest that allopurinol was able to reduce the activity of xanthine oxidase ending in reduction of chlorotic area in allopurinol treated plants compared to

untreated plants confirming Fujihara and Yamaguchi (1979) who reported that allopurinol inhibits the activity of xanthine oxidase.

There was a significant genotype × allopurinol interaction on chlorotic area indicating suggesting that the chlorotic area depended on the genotype and the level of allopurinol dosage. Despite Alsen and Ning7840 are contrasting genotypes that is Alsen lacks the *lm* gene while Ning7840 possesses the *lm* gene (Li and Bai, 2009), there was a general trend in the response of genotypes to the level of allopurinol application on chlorotic area. Higher levels of allopurinol (50µM and 75µM) treatment produced less chlorotic area (3536.00 ± 847.47, 1421 ± 847.47) compared to the control (12374.00 ± 847.47) and 25 µM (6520.00 ± 847.47) allopurinol dosages. Alsen × allopurinol treatment interactions also gave a similar trend, where high (50µM and 75µM) allopurinol dosage gave lower (499.94 ± 847.47 and 142.00± 847.47) chlorotic area compared with the low (25µM) and control allopurinol dosages that gave higher (1041.39 ± 847.47, 2141.22 ± 847.47) chlorotic area. The results agree to Berner and Van der Westhuizen (2010) findings, who reported a reduction in hypersensitive reaction symptoms in resistant cultivar of wheat to aphids in allopurinol treated plants compared to none treated plants. The hypersensitive system that include chitinase and peroxidase activities were reduced in allopurinol treated plants, making their resistant plants become susceptible. The suppression of xanthine oxidase activity and subsequently superoxide dismutase disrupted the hypersensitive defense system in the study, making plants susceptible to aphids. The same mechanism is similar to the observations made on Ning7840; allopurinol was able to disrupt xanthine oxidase, in turn inactivating superoxide peroxidase ultimately reducing the formation of chlorotic area. A reduction

in chlorotic area is the product of lack of hypersensitive responses that were inactivated allopurinol treatment.

The percent chlorotic area and number of lesions was higher in Ning7840 compared to Alsen genotypes since Ning7840 possesses a lesion mimic gene that produces lesion flecks from the booting stage unlike Alsen cultivar. There was a fivefold and fourfold difference in percent chlorotic area and number of lesions between Ning7840 and Alsen genotypes respectively. The huge variation in percent chlorotic area and number of lesions offers the opportunity to study effectively the contrasting genotypes. Berner and Van der Westhuizen (2010) used contrasting genotypes in elucidating the role of allopurinol in inhibiting xanthine oxidase activity against Russian Aphids in wheat. The contrasting genotypes were the resistant and susceptible lines. A study in Arabidopsis plants used RNA interference (RNAi) technique to create genotypes that contain genes that play a role in controlling the activities of xanthine dehydrogenase (XDX). This enzyme is significant in the oxidation of xanthine and hypoxanthine that catalyzes hypersensitive responses. The contrasting genotypes assisted in unravelling the role of xanthine dehydrogenase enzyme the uses of allopurinol chemical (Nakagawa et al. 2007). There was a reduction on percent chlorotic area and number of lesions with an increase in allopurinol level. The difference in percent chlorotic area and number of lesions between highest dosages to the control was thirteen and seven times suggesting that allopurinol was effective in inhibiting xanthine oxidase subsequently controlling the production of reactive oxygen species that are involved in hypersensitive responses. Higher levels of allopurinol were also able to control the disease symptoms on inoculated bean and wheat plants (Marte and Montalbini, 1999) since they displayed a delay in the

onset of symptoms, less number of lesions and pustules on the infection sites compared to the control. Furthermore, Montalbini (1994) showed that application of allopurinol in susceptible plants when inoculated with rust, they displayed resistance to pathogens by having reduced number and size of uredia and chitin quantity in the infected leaves. All these are the symptoms of reduced levels of hypersensitive reaction in the host induced by the reduction in the activity of xanthine oxidase.

The results also show that there was a genotype × allopurinol interaction on percent chlorotic area and number of lesions, suggesting that the effects of allopurinol depended on the genotype. Nevertheless, there was a trend in both genotypes where higher doses of allopurinol treatment gave lower percentage of chlorotic area and number of lesions. This trend suggests that higher dosage was superior in effectively inhibiting the activities of xanthine oxidase compared to lower dosages. Despite having a similar trend in responses between the Alsen × allopurinol and Ning7840 × allopurinol dosages, there was a huge variation percentage chlorotic area and number of lesions when comparisons were made between genotypes but maintaining allopurinol level. For instance, comparing Alsen × 75µM versus Ning7840 × 75µM, the former shows low percentage chlorotic area and lesion numbers compared to the latter. Similar observations were made on Alsen × 25µM versus Ning7840 × 25µM, where the latter had higher values than the former on lesion number and percent chlorotic area. The probable explanation for this scenario lies mainly in the genotype. First, the genotype Alsen does not possess the lm gene hence producing less number of lesions and percent chlorotic area at each dosage of allopurinol compared to Ning7840 that possesses the lm gene. The allopurinol dosage is the secondary factor that is applicable when comparing

the genotypes at different dosages. Higher values for the lesion number have been reported in many studies where they are used as a control against a set of dosages for studies. For instance, in a study of effects of allopurinol treatment in tobacco mosaic virus, the results showed that there was a difference in number of lesions in leaves between the untreated and treated plants 10 days post inoculation (Montalbini and Torre 1996). The results suggest that allopurinol is effective in suppressing the xanthine oxidase hence subsequently reducing the hypersensitive responses represented by a reduction in percent chlorotic area and number of lesions in allopurinol treated plants.

There was variability on chlorophyll content attributed to genotype, allopurinol dosage and genotype × allopurinol interaction. The results show that Ning7840 was superior to Alsen on chlorophyll content, the reason being that the green coloration in Ning7840 was deep as compared to Alsen cultivar. Allopurinol dosage had an effect on chlorophyll content, since higher dosage of allopurinol reduced the chlorophyll content compared to lower dosage of allopurinol and control treatments. A study involving silencing xanthine dehydrogenase (XDH) in Arabidopsis using RNAi silencing techniques revealed that genotypes whose XDH had been silenced completely suffered early an early accelerated onset of leaf senescence compared to the partially silenced and wildtype genotypes. The results suggest the role of the XDH in maintaining chlorophyll levels in leaves and reducing the onset of senescence (Nakagawa et al. 2007). Therefore, the findings in the study agree with the results on higher dosage of allopurinol that inhibit xanthine oxidase hence affecting chlorophyll synthesizing machinery. On the effect of genotype × allopurinol interaction on chlorophyll content, the variability was significant suggesting that genotypes varied on chlorophyll content in relation to the level of

allopurinol dosage. There was a reduction in chlorophyll content with an increase in allopurinol dosage in Ning7840 genotype. In Alsen, the scenario was different, where an increase in allopurinol dosage leads to a corresponding increase in chlorophyll content. The hypothesis that may illustrate the differences in response between the two genotypes lies in the genotype genome. Thus, Ning7840 possesses lm gene that induces the plant to constitutively produce the flecks during the booting stage unlike Alsen, which lacks the lm gene hence does not produce flecks. Application of allopurinol is effectively inhibiting the activity of xanthine oxidase hence inducing the early onset of senescence at higher rate in Ning7840 plant receiving high dosage of allopurinol. This is affecting the chlorophyll synthesizing machinery in Ning7840 plants under high allopurinol dosage hence having low values of chlorophyll content.

There were only allopurinol effects on number of heads but no genotype and genotype × allopurinol treatment interaction effects on number of heads. The non-significance on genotypic effects on number of heads suggests that the number of heads were biologically similar in both genotypes. The non-significance of genotype × allopurinol treatment interaction effects on number of heads suggests that all the treatment combinations performed equally regardless of the allopurinol or genotype on number of heads. However, allopurinol dose had an effect on the number of heads. The number of heads was higher under high dose of allopurinol but low under the control and 25µM allopurinol dosage. The results in the study contradict to the findings reported by Nakagawa et al. (2007), on RNAi xanthine dehydrogenase silenced Arabidopsis genotypes; where they reported that silencing xanthine dehydrogenase affected the fruiting bodies of Arabidopsis plants. Nevertheless, the results may suggest that the

plants under high dosage of allopurinol may have developed many heads as an adaptation to produce many seeds though majority of the seeds being infertile as was proposed by Went (1973).

There were significant allopurinol treatment effects on weight of heads but no significant genotype and genotype × allopurinol treatment interaction effects on weight of heads. The non-significance on the interaction and genotypes suggest that genotypes performed equally regardless of the allopurinol treatment. Furthermore, the weight of heads for Alsen genotype were similar to Ning7840 genotype due to absence of genotypic significance on the weight of heads. Allopurinol effects showed that the weight of heads was higher in the 75µM allopurinol dose than the 25µM. The results in the study disagree with observations reported by Nakagawa et al. (2007) on weight of fruiting bodies in partially and completely xanthine dehydrogenase genotypes of Arabidopsis. They reported that the partially and completely silenced genotypes had less and reduced fruiting bodies whose weight was also less compared to the wildtype. However, the comparison between the control and 25µM agrees with the results in Arabidopsis where the partially silenced xanthine dehydrogenase genotypes had marginally different weights of the fruiting bodies to the wildtype (Nakagawa et al. 2007).

Allopurinol treatment did not have an effect on the weight of stalks, but genotype and genotype × allopurinol treatment interactions had an effect on the weight of stalks. Lack of allopurinol treatment effect on the weight of stalks suggests that weight of stalks performed equally on all the levels of allopurinol, thus there was no net benefit on weight of stalks in growing the plants under allopurinol media. Genotypic effects showed that

Ning7840 was superior to Alsen on weight of stalks. Ning7840 was slightly taller and leafy compared to Alsen cultivar, hence producing heavier weight of stalks than Alsen. There was a significant genotype × allopurinol treatment interaction on weight of stalks, suggesting that the responses of genotypes on weight of stalks depended on dosage of allopurinol. When Ning7840 was the genotype, the control gave the less weight of stalks than the plants grown in allopurinol media. The results in the study contradict observations reported by Nakagawa et al. (2007) on Arabidopsis genotypes that were partially and completely silenced for xanthine dehydrogenase. The partially and completely silenced genotypes had less weight of biomass than the wildtype. Despite, the control having less weight of stalks compared to allopurinol treated plants, the margins in weight of stalks between the 50µM and 75µM allopurinol doses were minimal than with the 25µM allopurinol dose. Where Alsen was a genotype, the results show that there was minimal effect of allopurinol dose on the weight of stalks unlike in Ning7840. Thus, Alsen cultivar lacks the lesion mimic gene in its genome that minimizes the allopurinol effects on xanthine oxidase activity.

There was genotypic, allopurinol and genotype × allopurinol interaction effects on grain weight. On genotypes, Alsen gave more grain weight than Ning7840, the reason being that lesion mimics are generally low yielding than non-lesion mimic lines. The results agree with finding reported by Yao et al. (2009), where they observed wheat genotypes with lesion mimic gene gave lower yields than genotypes with no lesion mimic gene. There was variation on grain weight attributed to allopurinol dosage, with greatest variation observed between 50µM and 25µM levels with the former giving more yield than the latter. The hypothesis supporting this observation is that under lower dosage

rate, the allopurinol was not very efficient in inhibiting the xanthine oxidase activity as a result the plants were not able to partition more resources for grain filling than hypersensitive responses hence having less grain weight compared to the higher dosage (50µM). There was no variation between the control and highest allopurinol dosage, the suggested hypothesis being that under the control regime there was no xanthine oxidase inhibition as a result the plants could not prioritize resources for grain filling than hypersensitive responses. Under the high dosage regime, allopurinol was able to effectively inhibit xanthine oxidase but at the expense of affecting chlorophyll producing machinery that ultimately reduced photosynthates for grain filling. The effects of allopurinol in inhibiting xanthine oxidase and ultimately having reduced grain weigh are synonymous to the findings on use of RNAi technique in silencing xanthine dehydrogenase in Arabidopsis. The authors reported that the silenced genotypes had low seed weight, reduced fruit length and high numbers of fruit sterility compared to the wildtype in Arabidopsis plants (Nakagawa et al. 2007). Therefore, low grain weight under high allopurinol dosage may be a resultant implication on inhibiting the xanthine oxidase.

There was significant genotype × allopurinol interaction on grain weight suggesting that genotypic responses on grain weight varied with dosage of allopurinol. In general, Alsen × allopurinol interactions gave higher grain weight compared to Ning7840 × allopurinol combinations. The lesion mimic lines are generally low yielding than the non-lesion mimic lines (Yao et al. 2009) despite allopurinol treatment controlling the xanthine oxidase and subsequently reducing hypersensitive responses in Ning7840. There was a reduction in grain yield with an increase in allopurinol dosage, suggesting

there was a penalty cost associated with inhibiting the activity of xanthine oxidase as was observed with RNAi xanthine dehydrogenase silenced genotypes compared to the wildtypes in Arabidopsis plants (Yao et al. 2009). The authors reported that there was a reduction in in seed weight, increased number of sterile fruits and number of seeds per fruit in silenced genotypes compared to the wildtype. All these traits are highly correlated to grain weight.

There were significant genotypic and allopurinol but not genotype × allopurinol interaction on number of grains. Alsen a non-lesion mimic line was superior to Ning7840 on number of grains. Lesion mimic lines are less yielding as reported in wheat, barley, corn and rice by other authors, suggesting that our findings are in agreement with previous studies (Yao et al. 2009, Kjaer et al. 1990, Kinane and Jones 2000, Kamlofski et al. 2007 and Arase et al. 2000). There was no variability between the control and highest dosage of allopurinol on number of grains. In the case of control treatment, the hypersensitive responses were highest as a result the plant did not partition more photosynthates for grain filling hence having less number of grains. In case of highest dosage of allopurinol, the allopurinol effectively reduced the activity of xanthine oxidase reflected in reduced chlorotic area and number of lesions on leaves, but at a cost to grain filling. Inhibition of xanthine oxidase induces onset of leaf senescence (Nakagawa et al. 2007) hence affects chlorophyll machinery. Furthermore, Nakagawa et al. (2007) reported that in Arabidopsis, lines with xanthine dehydrogenase silenced, they produced many infertile seeds, less number of seeds compared to the wildtype genotypes. Similarly, higher dosage of allopurinol inhibited the activity of xanthine oxidase hence having same consequences on grain number produced compared to the control. Lack

genotype × allopurinol interaction suggests that the genotypes and allopurinol dosage combination performed equally on number of grains.

There was only significant genotypic variations on harvest index with non-significant allopurinol and genotype × allopurinol treatment interactions on harvest index. Alsen had a higher harvest index than Ning7840. Alsen cultivar does not possess a lesion mimic gene, produces many seeds compared to Ning7840, a mutant that has a lesion mimic gene. Furthermore, Yao et al. (2009) reported that lesion mimic varieties are less yielding than varieties that are not lesion mimics. The results on the weight of stalks showed that Ning7840 was superior to Alsen, but results on weight of grain showed that Alsen was superior to Ning7840. The results on weight of stalks and weight of grain support the results on harvest index, where harvest index normally is higher in cultivars that have low weight of stalks and high grain weight compared to a situation where the weight of stalks is higher than the weight of grain. Lack of allopurinol dose effect on harvest index suggests that there was no net benefit accrued to allopurinol application. There was no genotype × allopurinol treatment interaction on harvest index indicating that the treatment combinations performed the same resulting in producing similar harvest indices.

There were many significant strong positive correlations between many yield and yield components traits suggesting a strong association between the traits. Weight of grain was highly correlated to number of grain. In the study, there was a similar trend under the main effects of allopurinol and genotype × allopurinol interactions, where treatments that had high values for weight of grain had corresponding higher values for grain number. The results suggest that factors that affect number of grain automatically

affect the grain weight. Weight of heads was also highly correlated to weight of grain and number of grains. The hypothesis being that in the study the genotype × allopurinol interaction and main effects of allopurinol gave similar trends among the traits. For instance, at Alsen × 50µM treatment combination, the values were relatively high for both weight of heads and number of grains. Weight of stalks was highly correlated to number of heads, weight of heads, weight of grain and number of grain. The genotype × allopurinol interaction and the main effects associated with allopurinol dose gave similar trends on the responses corresponding to the values on the traits. Chlorotic area and percent chlorotic area were also strongly and positively correlated to number of lesions, suggesting that any attempts that reduce damages on the leaf automatically reduce the number of lesions on the photosynthetic apparatus. The results in the study revealed that that higher level of allopurinol was able to reduce the number of lesions, chlorotic area and ultimately reducing the percentage of the chlorotic area, creating a strong positive association between the traits. There was a weak but significant correlation between chlorophyll content and chlorotic area, percent chlorotic area and lesion number. The weak correlation may be attributed to the time difference when the chlorophyll data were collected to the time the leaf samples were quantified for chlorotic area, percent chlorotic area and number of lesions data. The chlorophyll content data were collected when the plants were at five leaf stage while the data for chlorotic and lesion number were collected when the plants were at the heading stage. Negative but significant correlations were observed between harvest index and total leaf area, percent chlorotic area, lesion number and chlorophyll content. The negative correlation suggests that these factors played a role in reducing the proportion of photosynthates whose sink was grain and

other plant parts. High percent chlorotic area and lesion number reduces the efficiency of photosynthetic apparatus that affects the quantity of photosynthates produced, ultimately reducing the amount for grain sink. Results from the study show that percent chlorotic area and number of lesion was highest under low and zero dosage of allopurinol dose. Under low and zero dose of allopurinol, xanthine oxidase was not effectively inhibited. As a result hypersensitive responses were high, the situation increased competition for photosynthates for defense as well as grain filling. A study by Kumudini et al., (2008) on soybeans revealed that high lesion number and loss in chlorophyll were factors that contributed to the loss in yield and harvest index. The results reported by Kumudini et al., (2008) are in agreement with the results in the study where low allopurinol dosage was associated with high values for lesion number and chlorotic area. Chlorophyll content also showed significant negative correlation with weight of stalks, weight of heads, weight of grain and number of grain. The results on correlation between chlorophyll content and weight of grain in the study disagrees with the results reported by Chang et al., (2015) where they reported a positive a correlation between chlorophyll content in the flag leaf to grain weight. Another study by Wang et al., (2008) also reported that high chlorophyll content in leaves increases the weight of biomass and grain yield in rice contradicting the observations made in the study where there was a negative correlation between chlorophyll content to weight of stalks, weight of heads and weight of grain. The suggested reason behind the negative correlation in the study compared to other studies may be attributed to timing when the chlorophyll data were collected. One study reported that chlorophyll data collected from sample plants at early stages of growth gave lower readings compared to readings collected in flag leaves of wheat

(Lawlor, et al. 1989). In the case of the chlorophyll data collected in the study, data were collected when plants had fives leaves hence fitting well with the observations reported by Lawlor et al., (1989), hence having a negative correlation to weight of stalks and grain weight.

2.8 Conclusion

Results from the study show that high dosage of allopurinol treatment was effective in inhibiting xanthine oxidase in Ning7840, a lesion mimic like of wheat. There was a reduction in chlorotic area, percent chlorotic area and number of lesions on leaf samples from plants under high regime of allopurinol chemical compared to a control and lower level of allopurinol treatments. The chlorotic area and lesions were the hypersensitive response symptoms of Ning7840 produced as a resultant of xanthine oxidase enzyme that produces reactive oxygen species. Reactive oxygen species being toxic kills the cells, producing the flecks/lesions, which are the typical symptoms of hypersensitive responses. The results also show that the effectiveness of allopurinol at high dosage in reducing the flecks in Ning7840 negatively reduces chlorophyll content and ultimately grain weight. Therefore, precautionary measures should be used when using allopurinol since high dosage has a penalty cost on grain weight. There is a need to quantify the xanthine oxidase production under different allopurinol dosages to ascertain the effectiveness of allopurinol compound in suppressing its activity since in our study we did not measure it.

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Table 2.1a: Analysis of variance for chlorotic area, percent chlorotic area, lesion number, chlorophyll content and number of heads

Effect	Chlorotic area (mm^2)	Percent chlorotic area	Number of lesions	Chlorophyll content (SPAD units)	Number of heads
Genotype	*P<.0001*	*P<.0001*	*P<.0001*	*P<.0001*	*0.1516*
Allopurinol	*P<.0001*	*P<.0001*	*P<.0001*	*0.0107*	*0.0114*
Geno × Allo	*P<.0001*	*P<.0001*	*P<.0001*	*P<.0001*	*0.1600*

Where Geno = genotype and Allo = Allopurinol level

Table 2.2b: Analysis of variance for weight of heads, weight of stalks, grain weight, number of grains and harvest index

Effect	Weight of heads (g)	Weight of stalks (g)	Weight of grain (g)	Number of grains	Harvest index
Genotype	*0.0858*	*P<.0001*	*P<.0001*	*P<.0001*	*P<.0001*
Allopurinol	*0.0127*	*0.8272*	*0.0009*	*0.0099*	*0.2287*
Geno × Allo	*0.1129*	*0.0016*	*0.0002*	*0.0674*	*0.1272*

Where Geno = genotype and Allo = Allopurinol level

Table 2.2a: Chlorotic area, percent chlorotic area, and number of lesions associated with genotype, allopurinol and genotype × allopurinol interaction.

Factor	Chlorotic area (mm^2)	Percent chlorotic area	Number of lesions
Genotype			
Alsen	943.64 ± 423.74b	2.49 ± 1.05b	18 ± 2.5b
Ning7840	5963.17 ± 423.74a	12.35 ± 1.05a	77 ± 2.5a
Allopurinol level			
0µM	7257.85 ± 599.25a	18.37 ± 1.49a	87 ± 3.56a
25µM	3780.94 ± 599.25b	6.52 ± 1.49b	55 ± 3.56b
50µM	1992.99 ± 599.25c	3.40 ± 1.49bc	33 ± 3.56c
75µM	781.83 ± 599.25c	1.41 ± 1.49c	13 ± 3.56d
Genotype × Allopurinol			
Alsen × 0µM	2141.22 ± 847.47cd	6.18 ± 2.10bc	44 ± 5.03c
Alsen × 25µM	1041.39 ± 847.47cd	2.49 ± 2.10c	16 ± 5.03de
Alsen × 50µM	499.94 ± 847.47d	0.97 ± 2.10c	10.± 5.03de
Alsen × 75µM	142.94 ± 847.47d	0.33 ± 2.10c	8 ± 5.03e
Ning7840 × 0µM	12374.00 ± 847.47a	30.53 ± 2.10a	133 ± 5.03a
Ning7840 × 25µM	6520.50 ± 847.47b	10.54 ± 2.10b	94 ± 5.03b
Ning7840 × 50µM	3536.03 ± 847.47c	5.84 ± 2.10bc	56 ± 5.03c
Ning7840 × 75µM	1421.67 ± 847.47d	2.48 ± 2.10c	23 ± 5.03d

Where the number to the right of ± represents the mean while to the left is the standard error.

Mean values with different letters are significantly different ($P < 0.05$, least significant difference).

Table 2.2b: Chlorophyll content, number of heads, and weight of heads associated with genotype, allopurinol and genotype × allopurinol interaction.

Factor	Chlorophyll content (SPAD Values)	Number of heads	Weight of heads (g)
Genotype			
Alsen	41.21 ± 0.26b	24 ± 0.79a	19.55 ± 0.71a
Ning7840	42.49 ± 0.26a	22 ± 0.79b	18.33 ± 0.71a
Allopurinol level			
0µM	42.21 ± 0.37a	22 ± 1.2ab	18.26 ± 0.99ab
25µM	42.21 ± 0.37a	21 ± 1.2b	17.37 ± 0.99b
50µM	41.86 ± 0.37ab	24 ± 1.2ab	19.92 ± 0.99ab
75µM	41.12 ± 0.37b	25 ± 1.2a	20.21 ± 0.99a
Genotype × Allopurinol			
Alsen × 0µM	40.71 ± 0.53cd	23 ± 1.6ab	18.44 ± 1.41ab
Alsen × 25µM	40.45 ± 0.53d	20 ± 1.6b	16.08 ± 1.41b
Alsen × 50µM	41.51 ± 0.53bcd	25 ± 1.6ab	21.59 ± 1.41a
Alsen × 75µM	42.18 ± 0.53abc	26 ± 1.6a	21.36 ± 1.41a
Ning7840 × 0µM	43.71 ± 0.53a	20 ± 1.6b	18.09 ± 1.41ab
Ning7840 × 25µM	43.28 ± 0.53a	22 ± 1.6ab	17.93 ± 1.41ab
Ning7840 × 50µM	42.91 ± 0.53ab	24 ± 1.6ab	18.24 ± 1.41ab
Ning7840 × 75µM	40.01 ± 0.53d	23 ± 1.6ab	19.06 ± 1.41ab

Where the number to the right of ± represents the mean while to the left is the standard error.

Mean values with different letters are significantly different ($P < 0.05$, least significant difference).

Table 2.2c: Weight of stalks, weight of grain and number of grains associated with genotype, allopurinol and genotype × allopurinol interaction.

Factor	Weight of stalks (g)	Weight of grain (g)	Number of grains
Genotype			
Alsen	35.82 ± 1.32b	13.18 ± 0.39a	472 ± 12.09a
Ning7840	44.44 ± 1.32a	10.19 ± 0.39b	397 ± 12.09b
Allopurinol level			
0µM	39.53 ± 1.87a	11.59 ± 0.56ab	440 ± 17.11ab
25µM	40.65 ± 1.87a	10.60 ± 0.56b	410 ± 17.11b
50µM	40.85 ± 1.87a	12.88 ± 0.56a	466 ± 17.11a
75µM	39.49 ± 1.87a	11.66 ± 0.56ab	424 ± 17.11ab
Genotype × Allopurinol			
Alsen × 0µM	39.06 ± 2.65bc	12.56 ± 0.79bc	459 ± 24.19ab
Alsen × 25µM	32.88 ± 2.65c	10.95 ± 0.79cd	436 ± 24.19bc
Alsen × 50µM	36.91 ± 2.65bc	15.05 ± 0.79a	511 ± 24.19a
Alsen × 75µM	34.45 ± 2.65c	14.17 ± 0.79ab	484 ± 24.19ab
Ning7840 × 0µM	39.99 ± 2.65bc	10.64 ± 0.79cd	420 ± 24.19bc
Ning7840 × 25µM	48.43 ± 2.65a	10.25 ± 0.79cd	385 ± 24.19c
Ning7840 × 50µM	44.79 ± 2.65ab	10.71 ± 0.79cd	420 ± 24.19bc
Ning7840 × 75µM	44.53 ± 2.65ab	9.15 ± 0.79d	363 ± 24.19c

Where the number to the right of ± represents the mean while to the left is the standard error.

Mean values with different letters are significantly different ($P < 0.05$, least significant difference).

Table 2.2d: Harvest index associated with genotype, allopurinol and genotype × allopurinol interaction.

Factor	Harvest index
Genotype	
Alsen	0.255 ± 0.007a
Ning7840	0.139 ± 0.007b
Allopurinol level	
0M	0.203 ± 0.009a
25µM	0.188 ± 0.009a
50µM	0.205 ± 0.009a
75µM	0.191 ± 0.009a
Genotype × Allopurinol	
Alsen × 0M	0.247 ± 0.014a
Alsen × 25µM	0.251 ± 0.014a
Alsen × 50µM	0.269 ± 0.014a
Alsen × 75µM	0.255 ± 0.014a
Ning7840 × 0M	0.159 ± 0.014b
Ning7840 × 25µM	0.126 ± 0.014b
Ning7840 × 50µM	0.142 ± 0.014b
Ning7840 × 75µM	0.126 ± 0.014b

Where the number to the right of ± represents the mean while to the left is the standard error.

Mean values with different letters are significantly different ($P < 0.05$, least significant difference).

Table 3: Correlations between traits in wheat

	ChloArea	PctChloAr	#Lesions	Chlorophy	WtStalks	#Heads	WtHeads	WtGrain	#Grain	H.Index
ChloArea	1.0000									
PctChloAr	0.8727	1.0000								
	<.0001									
#Lesions	0.7729	0.6893	1.0000							
	<.0001	*<.0001*								
Chlorophy	0.2222	0.2104	0.3009	1.0000						
	0.0001	*0.0003*	*<0.0001*							
WtStalks	0.1044	0.0398	0.1955	-0.1747	1.0000					
	0.0846ns	*0.5115ns*	*0.0011*	*0.0037*						
#Heads	0.0077	-0.0315	0.0397	-0.0967	0.8279	1.0000				
	0.8965ns	*0.5943ns*	*0.5107ns*	*0.1015ns*	*<.0001*					
WtHeads	0.0502	0.0144	0.0708	-0.1668	0.8908	0.9304	1.0000			
	0.3961ns	*0.8082ns*	*0.2311ns*	*0.0045*	*<.0001*	*<.0001*				
WtGrain	0.0276	0.0039	0.0349	-0.1963	0.8482	0.8786	0.9609	1.0000		
	0.6404ns	*0.9557ns*	*0.5612ns*	*0.0008*	*<.0001*	*<.0001*	*<.0001*			
#Grain	0.0464	0.0243	0.0659	-0.1879	0.8364	0.8869	0.9398	0.9717	1.0000	
	0.4332ns	*0.6818ns*	*0.2652ns*	*0.0014*	*<.0001*	*<.0001*	*<.0001*	*<.0001*		
H.Index	0.2381	-0.1694	-0.3292	-0.1623	-0.1032	0.2399	0.1934	0.3092	0.2988	1.0000
	<.0001	*0.0039*	*<.0001*	*0.0058*	*0.0881ns*	*<.0001*	*0.0010*	*<.0001*	*<.0001*	

Key: ChloArea = Chlorotic area, PctChloAr = Percent chlorotic area, #Lesions = Number of lesions, Chlorophy = Chlorophyll content, WtStalks = Weight of stalks, #Heads = Number of heads, WtHeads = Weight of heads, WtGrain = Weight of grains and H.Index = Harvest index.

Fig. Legends

Fig. 2.1. Genotypic effects on number of grains. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 2.2. Genotypic effects on harvest index. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 2.3. Allopurinol effects on number of heads. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 2.4. Allopurinol effects on weight of heads. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 2.5. Allopurinol effects on number of grains. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 2.6. Genotype × allopurinol interaction effects on chlorotic area. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 2.7. Genotype × allopurinol interaction effects on percent chlorotic area. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 2.8. Genotype × allopurinol interaction effects on number of lesions. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 2.9. Genotype × allopurinol interaction effects on chlorophyll content. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 2.10. Genotype × allopurinol interaction effects on weight of stalks. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 2.11. Genotype × allopurinol interaction effects on grain weight. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 2.12. Effect genotypic responses under different doses of allopurinol on number of lesions.

Fig. 2.1

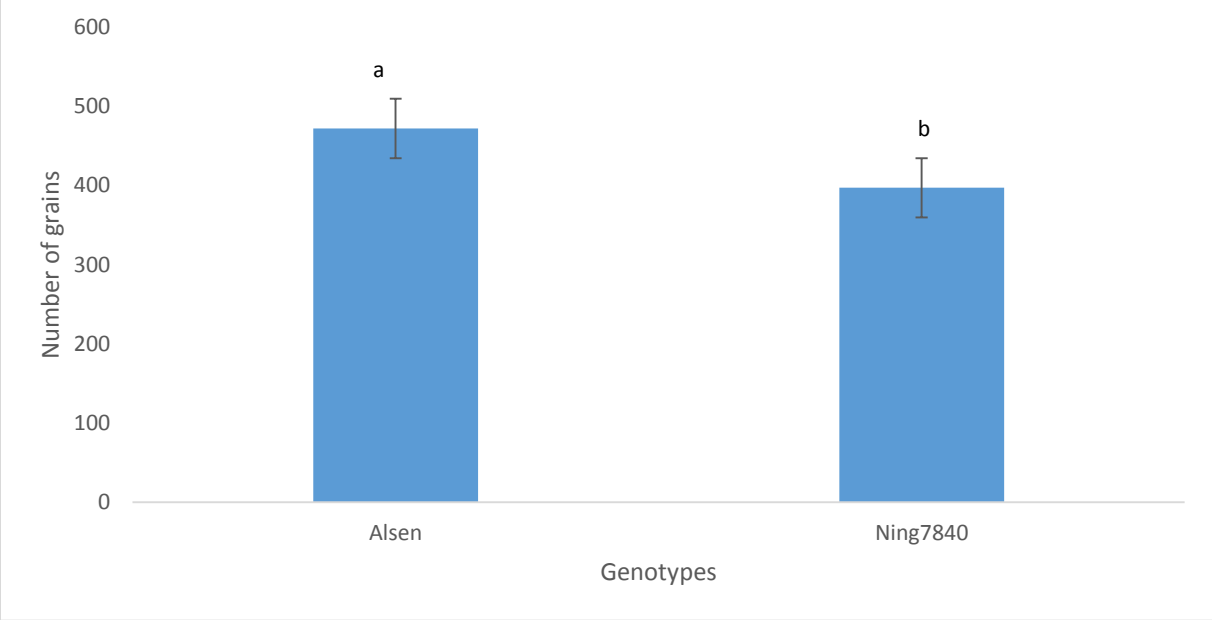


Fig. 2.2

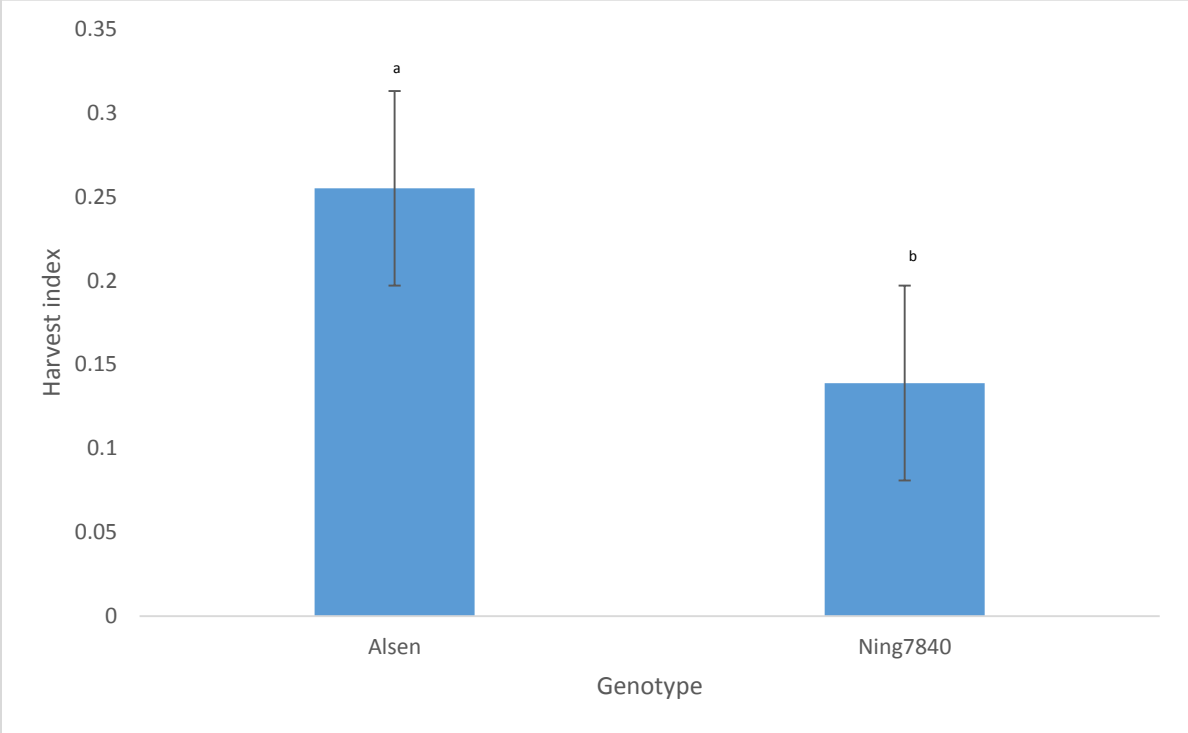

0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Harvest index
a
b
Alsen
Ning7840
Genotype

Fig. 2.3

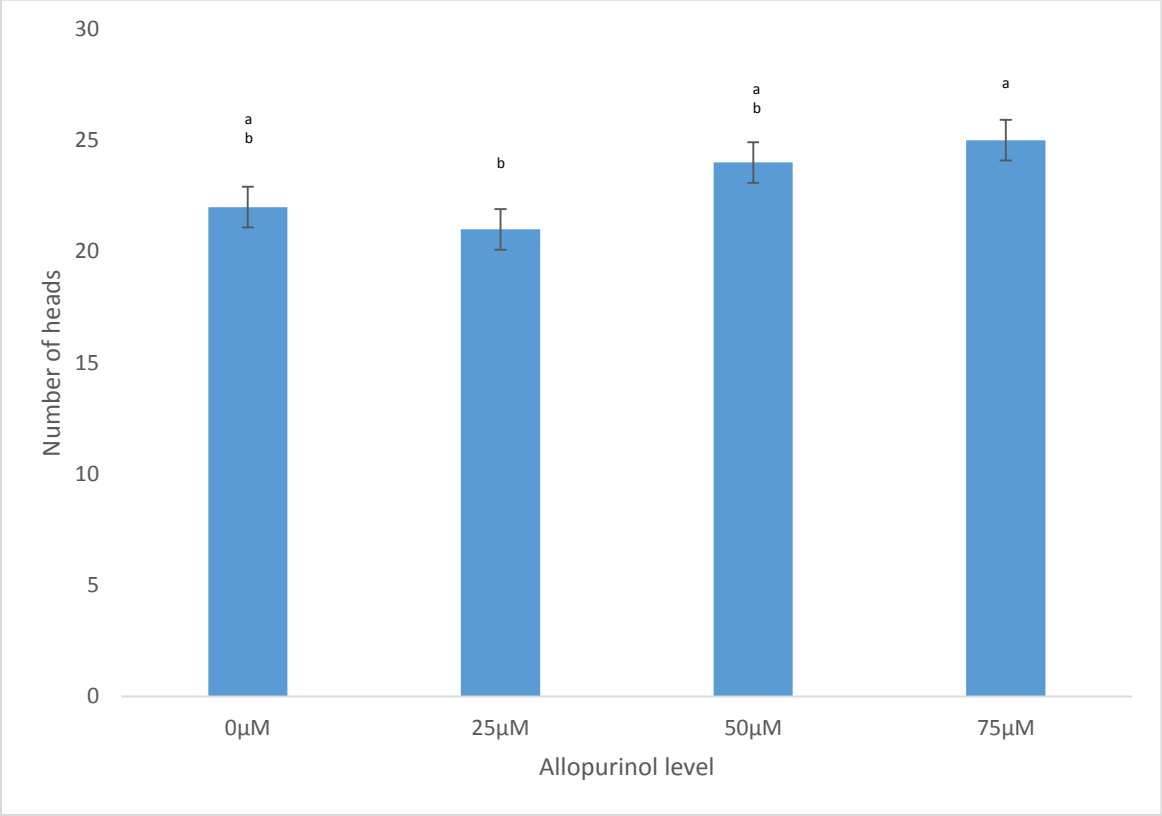

30
25
20
15
10
5
0
Number of heads
a
b
b
a
b
a
0µM
25µM
50µM
75µM
Allopurinol level

Fig. 2.4

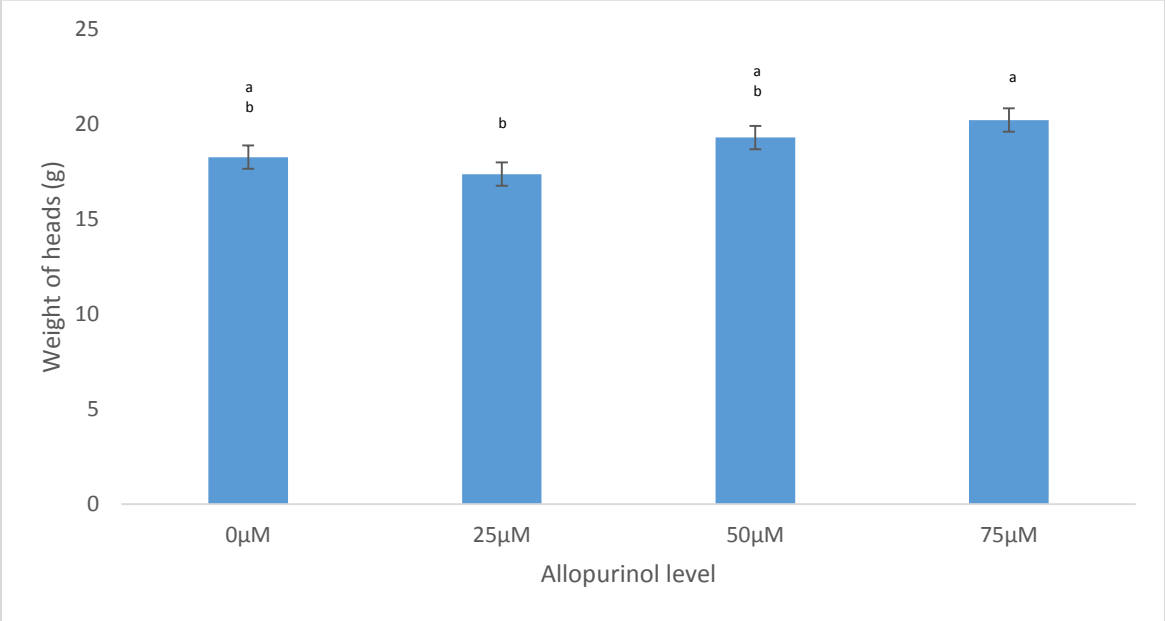

25
20
15
10
5
0
Weight of heads (g)
a
b
b
a
b
a
0µM
25µM
50µM
75µM
Allopurinol level

Fig. 2.5

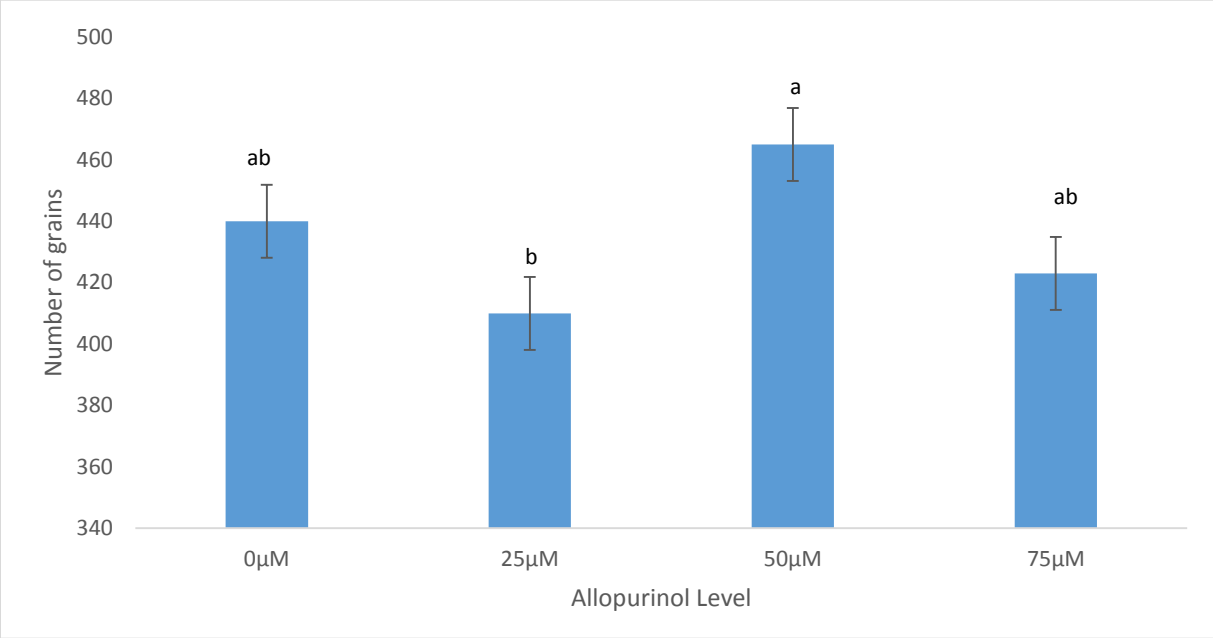

500
480
460
440
420
400
380
360
340
Number of grains
ab
b
a
ab
0µM
25µM
50µM
75µM
Allopurinol Level

Fig. 2.6

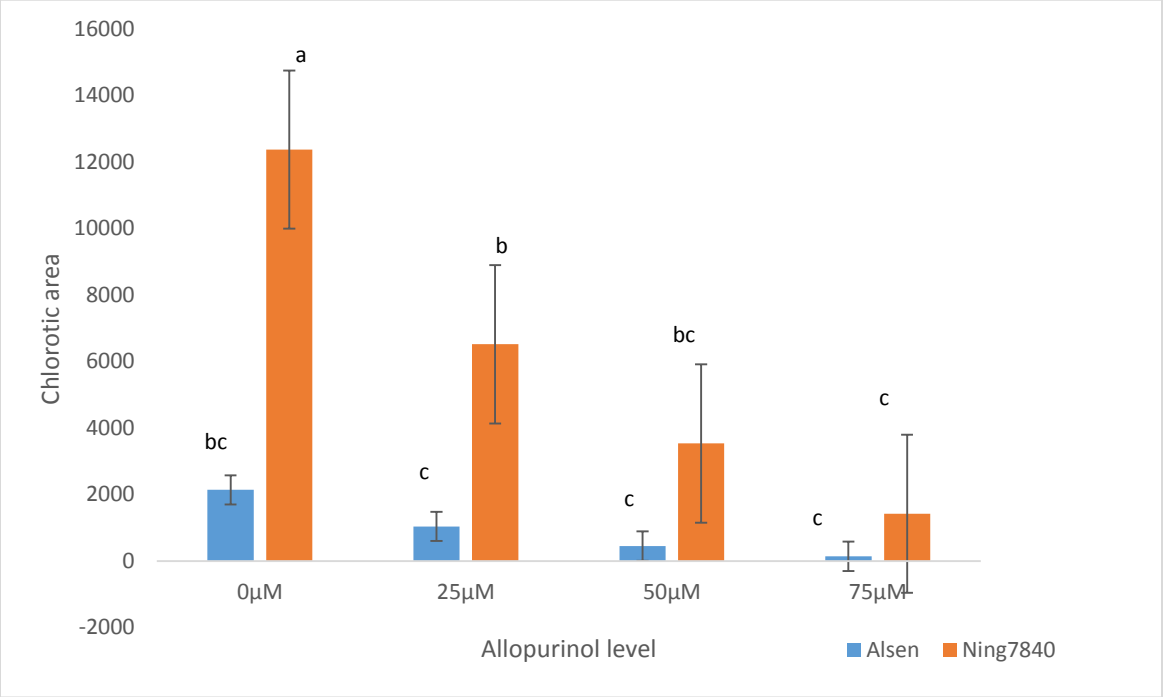

16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
Chlorotic area
a
b
bc
c
c
bc
c
c
0μM
25μM
50μM
75μM
Allopurinol level
Alsen
Ning7840

Fig. 2.7

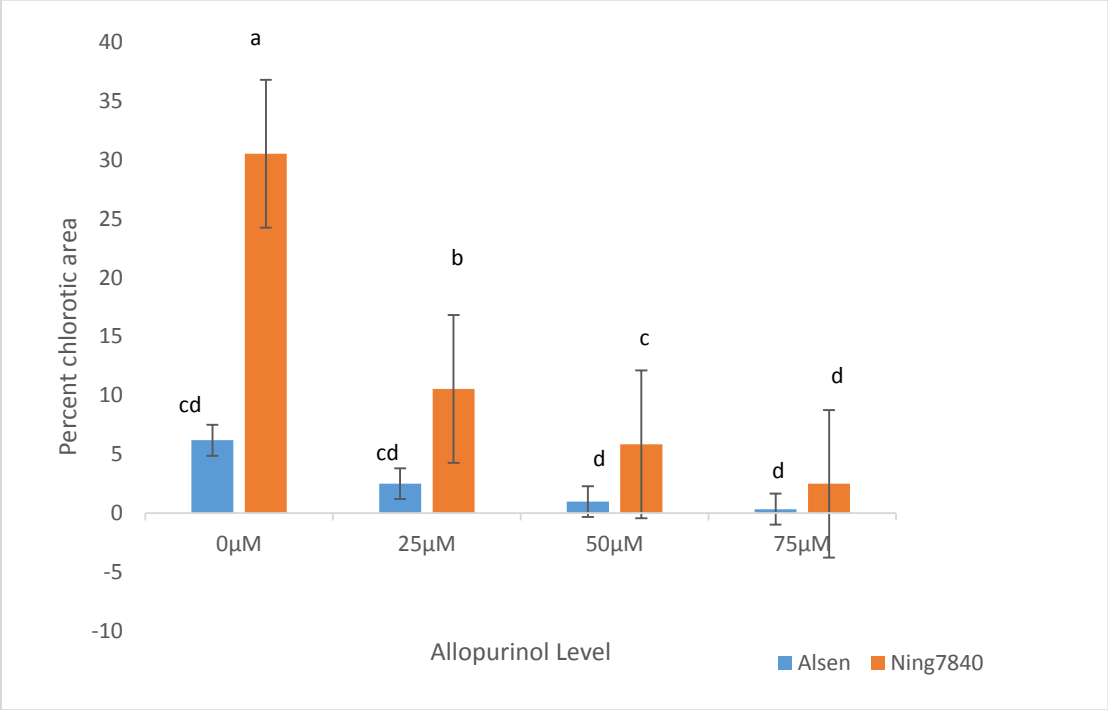

40
35
30
25
20
15
10
5
0
-5
-10
Percent chlorotic area
a
b
c
d
cd
cd
d
d
0µM
25µM
50µM
75µM
Allopurinol Level
Alsen
Ning7840

Fig. 2.8

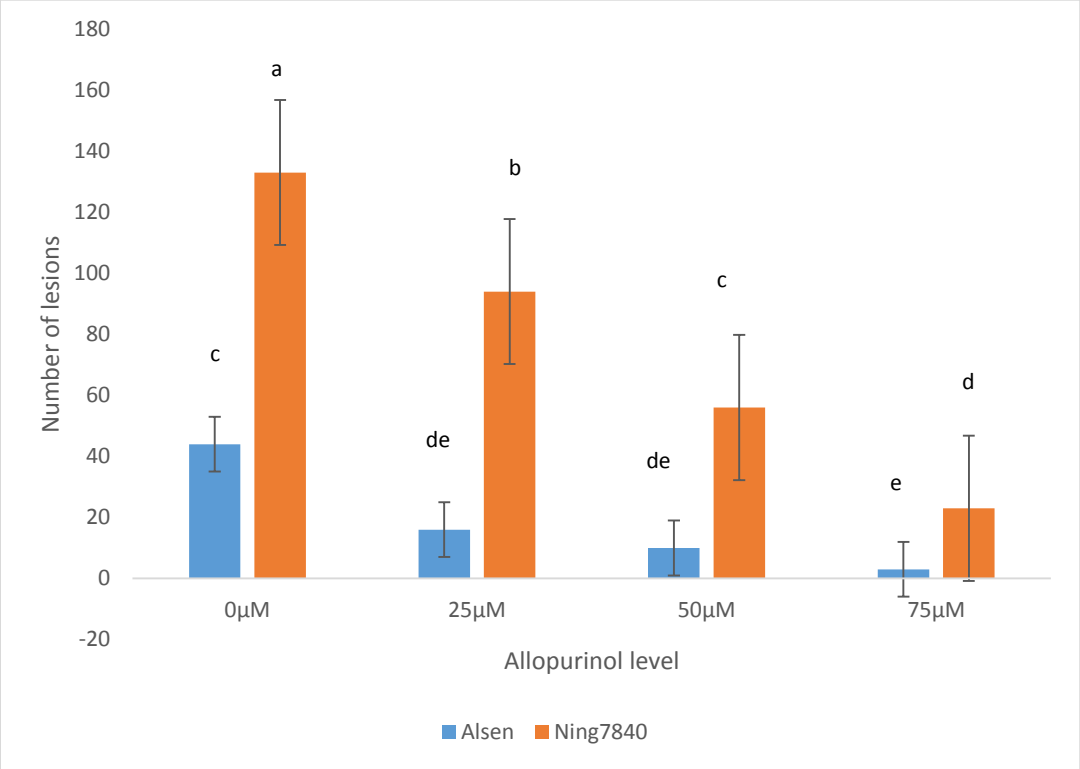

180
160
140
120
100
80
60
40
20
0
-20
Number of lesions
a
b
c
c
d
de
de
e
0μM
25μM
50μM
75μM
Allopurinol level
Alsen
Ning7840

Fig. 2.9

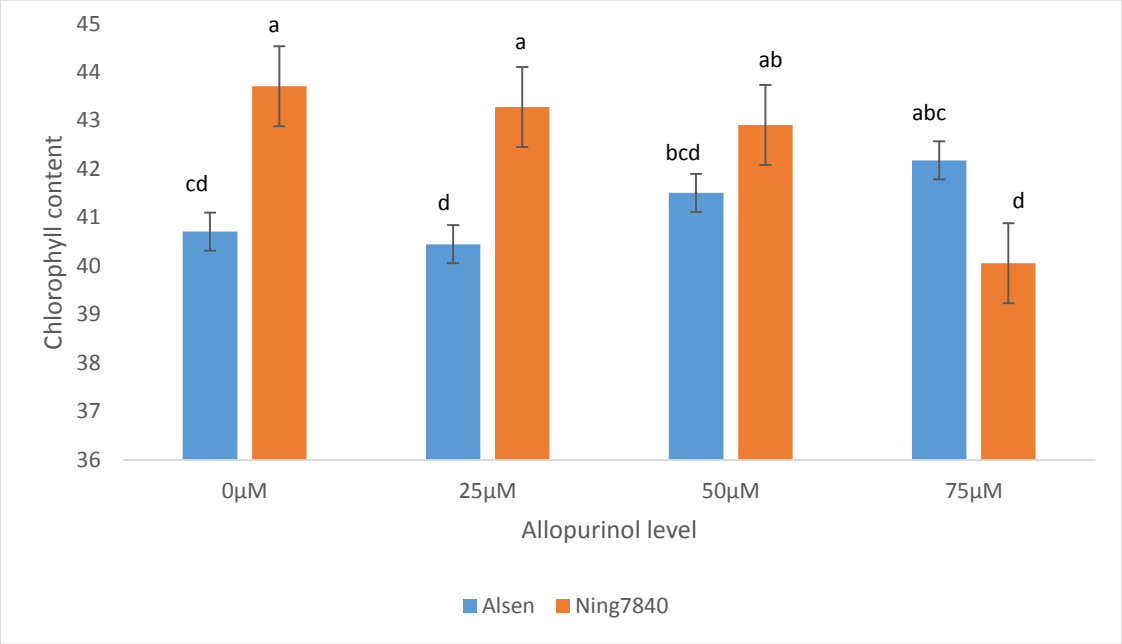

45
44
43
42
41
40
39
38
37
36
Chlorophyll content
cd
a
d
a
bcd
ab
abc
d
0μM
25μM
50μM
75μM
Allopurinol level
Alsen
Ning7840

Fig. 2.10

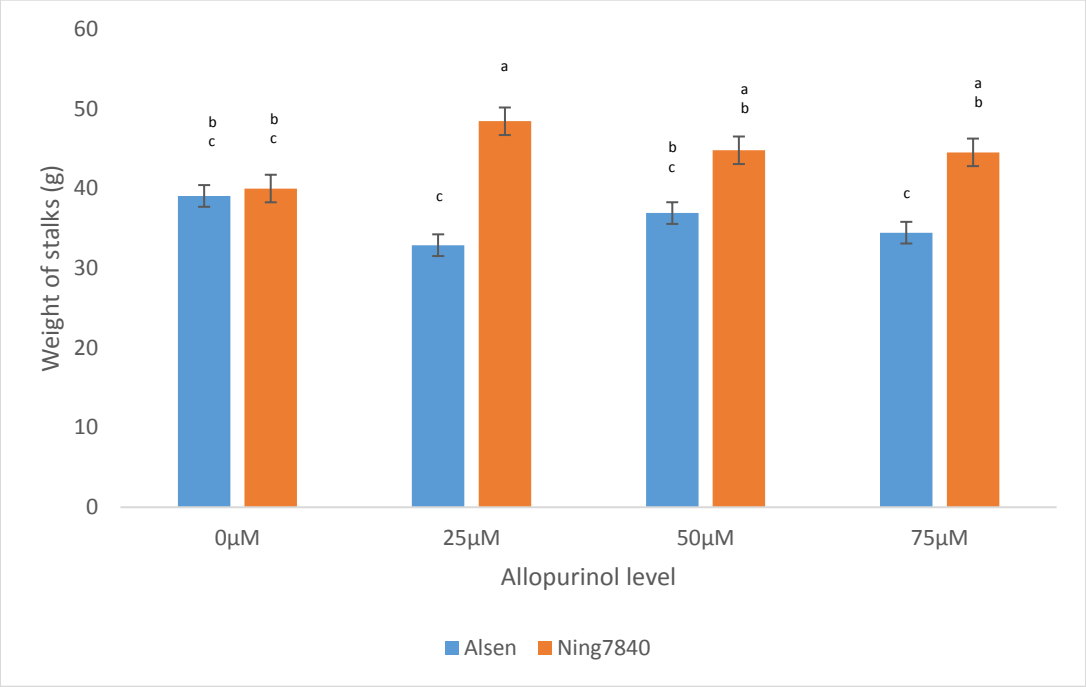

60
50
40
30
20
10
0
Weight of stalks (g)
b c
b c
c
a
b c
a b
c
a b
0μM
25μM
50μM
75μM
Allopurinol level
Alsen
Ning7840

Fig. 2.11

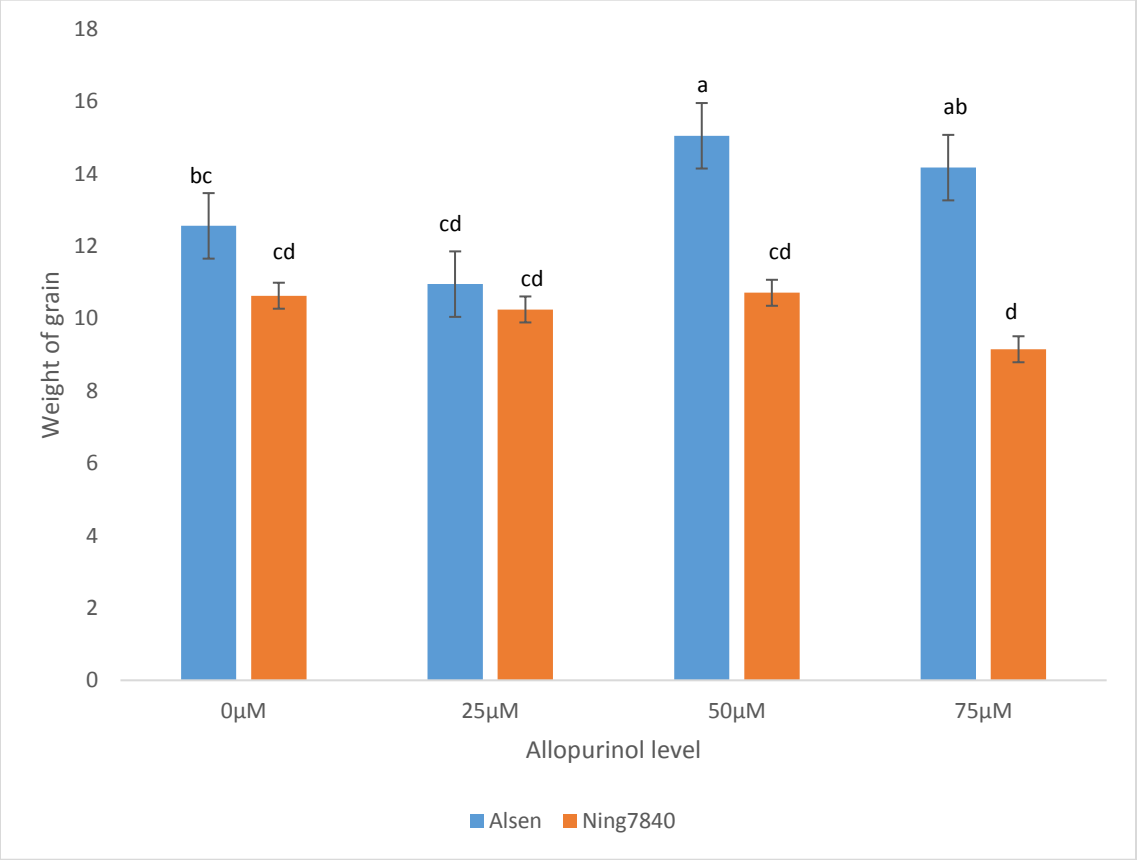
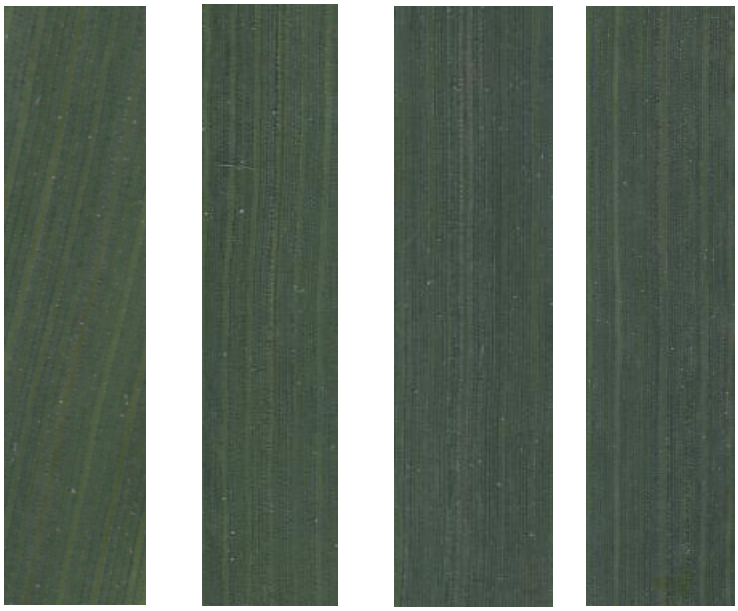
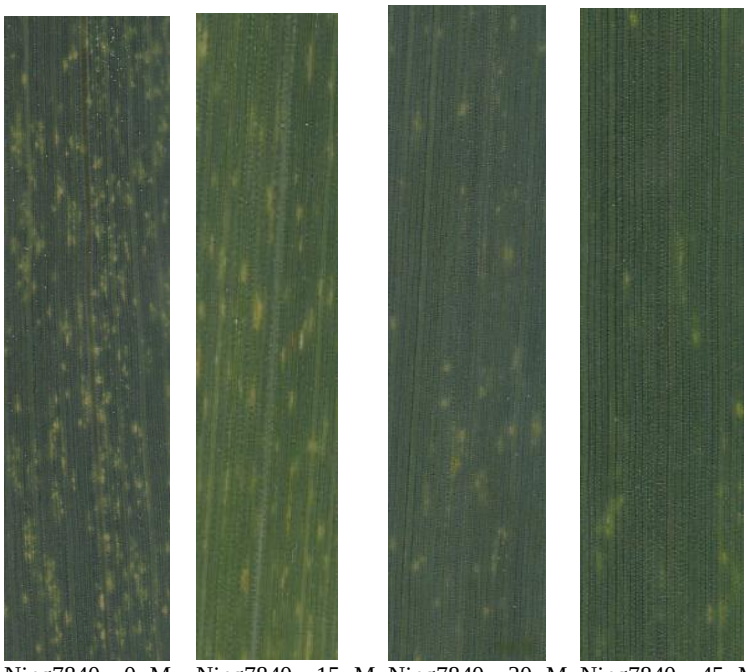

18
16
14
12
10
8
6
4
2
0
Weight of grain
bc
cd
cd
cd
a
cd
ab
d
0μM
25μM
50μM
75μM
Allopurinol level
Alsen
Ning7840

Fig. 2.12



Alsen x 0μM Alsen x 15μM Alsen x 30μM Alsen x 45μM



Ning7840 x 0μM Ning7840 x 15μM Ning7840 x 30μM Ning7840 x 45μM

CHAPTER 3: THE EFFECT OF ALLOPURINOL IN SUPPRESSING SUNFLOWER RUST (*Puccinia helianthi*) INFECTION IN SUNFLOWER (*Helianthus annuus* L.)

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3.1 Abstract

New races of sunflower rust can overcome R-genes in sunflower resulting in significant yield losses. Allopurinol has effectively reduced rust disease symptoms in other crops but it has not been applied to sunflower and sunflower rust pathogens. Different concentrations of allopurinol were applied as a soil drench to sunflower, and the higher concentrations of allopurinol reduced symptoms, but genotypes did not react differently. There was a reduction in number of seeds, weight of seeds, and weight of biomass with an increase in allopurinol concentrations. Allopurinol reduced rust symptoms, but this did not result in an increase in grain yield in comparison to the control.

Key words: *Helianthus annuus* L. *Puccinia helianthus* – Allopurinol – lesions, disease area, seed yield –seed number - biomass

3.2 Introduction

Sunflower (*Helianthus annuus* L.) is an industrial crop, produced globally and has multipurpose. Most of the commercial cultivars contain approximately 39 to 50 percent of oil content in the seed (NDSU Extension Service 2007). Furthermore, the seed oil is used for the production of vegetable oil, while the non-seed oil is used as human food and for bird market. Traits possessed by sunflower oil such as light color, high in unsaturated fatty acids, lack of linolenic acid, bland and high smoke points make sunflower oil fetch a premium price compared to other sources of vegetable oils (Luhs and Friedt 1994). Other studies have also revealed that sunflower oil may play a vital role in biodiesel since it has high monoalkyl esters made of long chains of fatty acids that increase the stability index in biodiesel fuels compared to other sources of vegetable oils (Moser, 2008). Sunflower oil may be used as a blend component or a substitute for other sources used in the biodiesel fuel production since it has low sulfur emissions (Moser, 2008). Commercialization of the technology may create several opportunities along the value chain in the sunflower industry.

Sunflower like any other plants face many challenges during their life cycle. The biotic and abiotic stresses if not controlled affect the normal physiological, molecular and biochemical processes in the plant ultimately reducing the crop productivity or killing the plant. Further classification within

biotic group results into either insect pests or diseases. Sunflower rust (*Puccinia helianthi*) is a foliar fungal disease that causes biological and economic yield losses if not controlled early in the season (Friskop et al. 2011, NDSU Extension Service, 2007 and Sendall et al. 2006). Four other Puccinia species namely *Puccinia canaliculata*, *Puccinia encleliae*, *Puccinia massalis* and *Puccinia xanthii* related to *Puccinia helianthi* cause rusts diseases in wild and cultivated sunflower plants (NDSU Extension Service 2007). *Puccinia helianthi* is a biotrophic suggesting that it requires a living host to survive (Kolmer et al. 2009). The pathogen is an autoecious and macrocyclic pathogen, thus it complete its lifecycle on a single host and produces different forms of spores during its life cycle respectively (Kolmer et al. 2009). The urediniospores are the most destructive stage of the fungi since they can cause multiple reinfections on the plant; attacking different parts of the plant including stems, leaves, bracts and petioles. Furthermore, wind and rain splashes carry the spores to other hosts causing new infections. As the season progresses, the urediniospores change to overwintering forms of spores to teliospores. Teliospores are dark colored, thick walled so that they resist environmental shocks such as cold and low moisture. The teliospores mark the end of the disease cycle for that particular season (Kolmer, et al. 2009 and NDSU Extension Service 2007). The cycle starts again in spring, when the telia germinate to basidia, which infects the plants and the cycle proceeds again. Temperatures of 55 to 85F coupled with high humid conditions favor the

multiplication of the pathogen. The multiplication cycle during the uredinial stage takes only 10 to 14 days to complete (Friskop, et al. 2011).

The presence of asexual and sexual reproduction in *Puccinia* pathogens offers opportunity for the pathogen to create new races of the pathogen through recombination (sexual) and increase the population of the new races through asexual reproduction. Sexual reproduction occurs when basidiospores produce the haploid gametes, which recombine forming aeciospores. There is multiplication of new races during the uredinial stage and teliospore stages (Kolmer et al. 2009, Groth and Roelfs 1982, Groth et al. 1995and Carvalho et al. 2011). The presence of asexual reproduction poses complex challenges in cultivar development for resistance against fungal pathogens since new strains of pathogens emerge all the time. In sunflower, studies have reported the presence of different isolates of sunflower plants (Friskop, et al. 2015) suggesting new virulent strains are emerging all the time (Kong and Kochman 1996). Furthermore, studies also show that some strains are able to cause infection in several differential lines such as 777, 776, 737, 376, 337 and 336 isolates (Friskop et al. 2015). Use of resistant genes is one the tools available in managing the disease. Many elite sunflower cultivars possess these resistant genes, such as R_1 (mc90), R_2+R_{10} (mc29), R_{4e} (P386), R_{4a} (HA-R1), R_5 (HA-R2), R_{4b} (HA-R3), R_{4c} (HA-R4), R_{4d} (HA-R5) (Friskop et al. 2015) and HAR-6 (Bulos et al. 2013). Nevertheless, use monogenic resistance in hybrids limit the lifetime of the

commercial hybrids since the selection pressure for the isolate intensifies, resulting into generation of more virulent strains. Another approach involves the sequential addition of resistant genes in the already elite lines through gene pyramiding. Though the process takes a long time to add a set of R-genes using tradition breeding approaches, use of DNA based markers offers opportunities to fast track the process. Lawson et al. (1998) reported using sequence characterized amplified region (SCAR) a marker assisted selection procedure in identifying two genes that confer resistance against sunflower rust. Using DNA based markers, offer opportunities in screening the lines at an early stage of the breeding program, before inoculation treatments hence may assist in reducing the costs associated with screening large numbers of materials as happens in conventional line development (Xu and Crouch, 2008 and NDSU Extension Service 2007).

Other tools available to farming community that assists in controlling sunflower includes foliar application of with fungicides. The objective of foliar application is to limit occurrence of new infections in the process slow down disease development from forming epidemics. Timely application at appropriate dosage and the right fungicides to the plants should be the key to success pathogen control (Friskop et al. 2011and Friskop et al. 2015). On the hand, the fungicides if not properly managed may promote selection for virulence in the pathogen, being poisonous are hazardous to people and animals and its application is dependent on prevailing environmental conditions. For instance,

the effectiveness of the fungicide may be reduced if soon after its application the location receives rainfall. If the application day or time coincides with windy or rainy weather conditions then famer cancels the application schedule. Crop rotation may assist in breaking the pathogen life cycle since the emerging spores may fail to infect the non-related crop to sunflower hence disrupting the rust incidences. Crop rotation may also reduce the development of race changes and occurrence of epidemics in sunflower growing environments (Friskop et al. 2011and Fetch et al. 2011). Another approach involves controlling the reservoir of the Puccinia within the location of sunflower fields. The reservoir includes the wild relatives of sunflower plants and all the volunteer plants, since the basidiospores may kick start the life cycle in these plants and increase the multiplication of the urediniospores in these plants before they spread to sunflower plants causing epidemics.

Allopurinol has the chemical formulae [4-hydroxypyrazolo (3,4-d) pyrimidine] is used in the medical field treating multiple ailments that includes tissue and vascular associated injuries, inflammatory, gout and heart failure associated diseases (Ng, et al. 2014 and Pacher et al 2006). Allopurinol is an isomer of hypoxanthine and some authors have reported that it inhibits activities of xanthine oxidase (Massey et al. 1970, Spencer and Johns 1970 and Fujihara and Yamaguchi 1978). Nakagawa et al. (2007) used RNAi technic in generating partially and completely xanthine dehydrogenase silenced Arabidopsis genotypes,

where the partially and completely silenced genotypes expressed the symptoms similar to the ones expressed when Allopurinol is applied to plants. Other studies have reported the purine decomposition is inhibited when germinating seeds, roots and suspension culture cells absorb Allopurinol chemical, indicating the effect of Allopurinol chemical on xanthine oxidase (Della Torre and Montalbini, 1995 and Montalbini and Della Torre, 1995). Allopurinol has been reported to reduce the pathogenic activity when susceptible plants treated with allopurinol were inoculated with pathogens. For instance, in tobacco when susceptible lines received a dosage of allopurinol, the disease symptoms of tobacco necrosis virus and the quantity of virus titres were reduced compared to the control (Montalbini and Torre, 1996). Montalbini and Della Torre, (1995) identified that the oxypurinol and ribonucleosides were the metabolic products of allopurinol in plants which were responsible for preventing tobacco necrotic virus hypersensitive responses and infection process induced by tobacco necrotic virus particles in allopurinol treated plants (Montalbini & Torre, 1996). In wheat crop, studies involving compatible and incompatible pathogen-host interactions treated with allopurinol, contrasting responses were observed. Under compatible reaction, the allopurinol treated plants showed hypersensitive responses and the intensity was proportional to the allopurinol dosage. Furthermore, the level of symptoms of pathogen growth and disease development declined with increase in level of allopurinol dosage. The results suggest that allopurinol created non-conducive

environment for pathogen establishment and spread in the susceptible host. On the contrary, under incompatible host-pathogen interaction allopurinol dosage did not change the hypersensitive responses on the host, though the number of lesions was reduced at higher levels indicating the effects of allopurinol chemical (Ádám et al. 2000) in reducing the pathogenic establishment and growth in the host. Furthermore, the study also reported an increase in xanthine metabolites in allopurinol treated plants, which is an indication of xanthine oxidase inhibition. In a study involving Russian Wheat Aphids on a resistant cultivar of wheat, the allopurinol-treated resistant plants recorded low levels of hydrogen peroxide, chitinase and reactive oxygen species because allopurinol chemical inhibited the activity of xanthine oxidase (Berner and Van der Westhuizen, 2010).

Reactive oxygen species (ROS) plays a fundamental role in hypersensitive responses; studies indicate that its toxicity is associated with death of cells (Auten and Davis, 2009, Kimura et al. 2005). Reactive oxygen species production has been reported in interactions involving pathogens like viruses, fungi, bacteria and insects with host plants because of induction of hypersensitive responses. Studies show that ROS is produced from enzyme NAD(P)H oxidase located in the plasmalemma or by peroxidase enzyme located in the apoplastic space and cell wall (Vanacker et al. 1998 and Bestwick et al. 1997). Xanthine oxidase localized in the peroxisomal matrix has been implicated as the source of ROS and is involved in the breakdown of purine compounds into nucleic acids and

nucleotides that includes guanine and adenine (Zrenner et al. 2006). Furthermore, xanthine oxidases localized in glyoxisomes are also involved in the synthesis of ROS (Montalbini, 1995, Sandalio et al. 1988, Del Rio et al. 1989 and Harrison 2002). Studies involving tobacco plants when inoculated with tobacco mosaic virus, rust inoculated studies in tobacco, wheat and beans all indicated that xanthine oxidase was the source for ROS (Montalbini and Della Torre, 1995 and Montalbini, 1992). On the other hand, under incompatible reaction in beans Montalbini, (1992) reported that allopurinol treatment suppressed hypersensitivity responses and there was an increase in electrolyte leakage. Reactive oxygen species increases the activities of enzyme lipoxygenase under rust infection in oats (Yamamoto and Tani, 1986) and in wheat, when inoculated with stem rust; there is an increase in lipid peroxidation (Abdou et al. 1993).

Use of allopurinol has been reported to be effective in controlling pathogen infection in some crops at a pilot phase, but none has been tested on sunflower rust. Therefore, they study wanted to evaluate if the same ROS are involved as in disease responses and if reducing lesions on leaves may reduce plant stress and lead to improved plant production.

3.3 Materials and Methods

Plant material: BC_4F_1 Near Isogenic lines (NILs) were generated by crosses from different R gene sources namely cm90, HAR2 and HAR3 crossed to a susceptible line HA89. The F_1 progeny was backcrossed to the HA89 four times until the BC_4F_1 seed was generated. The designated lines were HA89*5/cm90, HA89*5/HAR2, HA89*5/HAR3 and a check HA89, which is a susceptible line.

A split plot experiment in a randomized complete block design with races as a main block, genotype and allopurinol level as a subplot, replicated three times was utilized. The races used were 304 and 336 with no inoculation as a control. There levels for the genotypes and allopurinol were four and four respectively. The levels for allopurinol were 0µM (control), 25µM, 50µM and 100µM respectively. Two seeds of each cultivar were sown in 126 pots whose dimensions were 7″ (height) X 6″ (top diameter), and thinned to one seedling at five days post emergence. The potting media comprised one part loam soil and three parts Sunshine Mix-1(Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada). Incorporation of the loam soil assisted in water retention in the pots. Pots were placed on plates to collect excess drainage from the pots soon after irrigating the plants. Each treatment combination comprised of two pots in each replicate.

Plants were inoculated with rust races at 18 days after emergence. The races were mixed with sorbitol solution before spraying on the leaves. Ten minutes after spraying the inoculum on the leaves, a fine mist of water was sprayed on the leaves to dissolve excess salts from the sorbitol solution. Plants were placed in a modified growth chamber, made of plastic bags filled with some water then tied at the top. The concept was to create humid and high temperature conditions that aid the infection process of the pathogen on a host. The inoculation process took place in the evening, the lights in the greenhouse turned off creating a dark condition. The pathogen requires dark condition for infection process to proceed in a host. Plants were removed from the plastic bags 12 hours later, placed on the trays and irrigated with respective allopurinol solution. 250ml of each irrigation solution treatment was applied in specified pots using a soil drench method. Irrigation amounts were increased to 500ml and 1000ml per pot from 90cm height and head filling stage of plant growth. Irrigation frequencies were also increased from twice to four times each week as the demand for water from the plants was higher with age. At five weeks post emergence, 5g of N 14: P 14: K16 + minors Multicote 4 controlled release fertilizer (Haifa Nutri. Tech. Cranes Roots Blvd, Altamonte Springs, Florida, USA) was applied to each pot. The experiments were implemented during late fall to mid-winter season under greenhouse conditions where temperatures were maintained at 25 ± 3^{o}C with a 16

hour day length maintained with supplemental lighting. The experiment was repeated two times.

Data collection: At 14 days-post-infection disease rating was done on all inoculated blocks using a Sunflower Rust Disease Severity Rating Score developed by North Dakota State University (Friskop et al., 2011). Inoculated leaf samples were also scanned at 14 days post-infection where two leaves were randomly selected from each pot. Furthermore, two leaves were also randomly selected from the non-inoculated block, which were at the same stage when the inoculation treatment was applied in other blocks. The leaves from the non-inoculated block were also scanned. A Canon Scan LiDE 120 (Canon Latin America, One Canon Park, Melville, NY, USA) scanner connected to a laptop computer captured the samples and stored in form of TIF picture format in the laptop for further quantification. ASSESS 2.0 Image Analysis Software for Plant Disease Quantification (The American Phytopathological Society, Pilot Knob Road, St. Paul, MN, USA) package was used to quantify the pictures for total leaf area from the sampled leaf, chlorotic area, percent chlorotic area and lesion count.

Plants were harvested after attaining physiological maturity, yield and yield components data were collected. Data collected included number of heads, weight of heads, above ground biomass, weight of seeds and number of seeds. A

seed counter (Davis Tool and Engineering Inc. Manufacturers, Montgomery, Illinois, USA) was used to count number of seeds from each pot.

Statistical analysis: Generalized linear mixed model approaches were used to analyze the data using the PROC MIXED routine in SAS (v9.4; SAS Institute Inc., Cary, NC), where number of runs, blocks and replicates were treated as random, while genotype and allopurinol level were treated as fixed factors. The data were tested at 0.05 alpha level to determine mean significance.

3.4 Results

3.4.1 Race main effects

There were significant differences attributed to race on percent disease area, number of lesions, NDSU rating score, days to maturity, weight of stalks (g) (Table 3.1a), area of head (cm^2), number of seeds, weight of seeds (g), weight of the biomass (g) and harvest index (Table 3.1b) ($P<.05$). However, there were no significant differences on plant height (cm) (Table 3.1a) and weight of head (g) (Table 3.1b) attributed to race ($P>.05$). The highest percent disease area was observed on plants inoculated with race 336 (9.03 ± 0.389) followed by 304 (2.83± 0.389) race, while the control gave the least percent disease area (0.01 ± 0.389) (Table 3.2a). A similar trend was observed on number of lesions, where plants inoculated with 336 and 304 races had 38 ± 7.6 and 26 ± 7.6 mean number of lesions respectively, while the control had the least number **(**05 ± 7.6) of lesions (Table 3.2a). Rating the severity of sunflower rust infection using the NDSU Sunflower rating score showed that the infection was higher with 336 race (1.106 ± 0.114) compared to 304 (0.819 ± 0.114), while the control gave the least score (0.005 ± 0.114) (Table 3.2a). The results suggests that there was variation on rate of infection on the host attributed by races as reflected by the differences on percent disease area, number of lesions and the NDSU rating score. There was no variation in number of days to maturity between the control and the plants

inoculated with race 304. However, the plants inoculated with race 336 were significantly different from the control and plants inoculated with 304 race. Thus, the plants inoculated with race 336 attained physiological maturity earlier (121 ± 0.67) than the control (123 ± 0.67) and race 304 (124 ± 0.67) inoculated plants (Table 3.2a).

There was variability on weight of stalks (g) attributed to race, with the plants inoculated with race 336 had the highest weight (37.21 ± 0.56) followed by the non-inoculated plants (33. 78 ± 0.56) while plants inoculated with race 304 had the lowest weight (31.96 ± 0.56) (Table 3.2b). There was no variation on area of head (cm^2) between the control (55.96 ± 1.30) and plants inoculated with race 336 (57.81 ± 1.30) although the two treatment were significantly different from plants inoculated with race 304 (50.18 ± 1.30) that gave the smallest area of head (Table 3.2b).

There was a variation in the number of seeds attributed to race. Plants inoculated with race 336 had more seeds (331 ± 8.81) than the plants that were not inoculated (309 ± 8.81), while the plants inoculated with race 304 had the least number of seeds (264 ± 8.81) (Table 3.2c). There was no variability on weight of seed (g) between the control (12.41 ± 0.31) and plants inoculated with race 336 (12.35 ± 0.31). However the plants inoculated with race 304 had the least weight of seeds (10.72 ± 0.31) and were significantly different from the

control and plants inoculated with race 336 (Table 3.2c). There were significant variations on weight of biomass (g) due to effects of race. Plants inoculated with race 336 had the highest weight of biomass (62.88 ± 0.98) followed by the control (59.34 ± 098) and the plants inoculated with race 304 had the least weight of biomass (56.05 ± 0.98) (Table 3.2c). The only variation on harvest index attributed to race was between the control (0.187 ± 0.007) and plants inoculated with race 304 (0.166 ± 0.007). There were no significant difference on harvest index between the control (0.187 ± 0.007) and the plants inoculated with race 336 (0.178 ± 0.007). Furthermore there was no variability on harvest index between plants inoculated with race 304 (0.166 ± 0.007) and plants inoculated with race 306 (0.178 ± 0.007) (Table 3.2c).

3.4.2 Genotypic main effects

Significant genotypic variability effects were observed on plant height (cm) (Table 3.1a), number of seeds and weight of seeds (g) (Table 3.1b) ($P<.05$). There were no significant genotypic effects on percent disease area, number of lesions, NDSU rating score, days to maturity, weight of stalks (g) (Table 3.1a) area of head (cm^2) , weight of head (g), weight of the biomass (g) and harvest index (Table 3.1b) ($P>0.05$). Significant variations on plant height (cm) were observed between HA89*5/HAR3 (129.29 ± 13.0) and HA89*5/HAR2 (119 ± 13.0), HA89*5/HAR3 (129.29 ± 13.0) and HA89 (123.21 ± 13.0). However,

there were no variations in plant height between HA89 (123.21 ± 13.0) and HA89*5/cm90 (126.27 ± 13.0). Furthermore, there was no variability on plant height between HA89*5/HAR2 (119.59 ± 13.0) and HA89 (123.21 ± 13.0) (Table 3.2b). Genotypic differences on number of seeds were observed between HA89*5/HAR2 (277 ± 10.17) and HA89*5/HAR3 (330 ± 10.17) genotypes, HA89*5/HAR2 (277 ± 10.17) and HA89*5/cm90 (312 ± 10.17) and finally between HA89*5/HAR3 (330 ± 10.17) and HA89 (287 ± 10.17). The range in number of seeds ranged from 277 ± 10.17 to 330 ± 10.17. HA89*5/HAR3 produced highest number of seeds (330 ± 10.17) compared to the rest of genotypes (Table 3.2c).

Significant variation on weight of seeds (g) was observed between HA89*5/HAR3 (12.47 ± 0.36) and HA89*5/HAR2 (11.12 ± 0.36). However, there was minimal seed weight variability among HA89*5/HAR2 (11.12 ± 0.36), HA89*5/cm90 (11.83 ± 0.36) and HA89 (11.88 ± 0.36) respectively. Furthermore, there was significant variation on seed weight among HA89*5/HAR3 (12.47 ± 0.36), HA89*5/cm90 (11.83 ± 0.36) and HA89 (11.88 ± 0.36) genotypes (Table 3.2c). HA89*5/HAR3 produced more seed quantity than the rest of the genotypes.

There was no genotypic variation on percent disease area among the genotypes. The percent disease area ranged from 3.16 ± 0.445 to 4.65 ± 0.445 for

genotypes HA89*5/cm90 and HA89*5/HAR3 respectively (Table 3.2a). There was no variation on number of lesions attributed to genotypes. The number of lesions ranged from 21.29 ± 7.67 (HA89*5/HAR2) to 23.66 ± 7.67 (HA89*5/cm90) (Table 3.2a). There was no variation on NDSU disease rating score on genotypes; the disease rating scores ranged from 0.552 ± 0.122 to 0.714 ± 0.122 for HA89*5/HAR2 and HA89*5/cm90 respectively (Table 3.2a). There was no significant genetic variation on number of days to physiological maturity. The number of days ranged from 122 ± 1.21 (HA89*5/cm90) to 124 ± 1.21 (HA89) (Table 3.2a). Despite insignificant variability on weight of stalks (g) between genotypes, HA89*5/cm90 had the highest weight of stalks (34.71 ± 0.65) while HA89*5/HAR2 had the least weight of stalks (33.72 ± 0.65) (Table 3.2b). Insignificant variability was observed on area of head (cm^2) between genotypes. HA89 had the biggest area of head (55.59 ± 1.50) while HA89*5/cm90 had the smallest area of head (52.76 ± 1.50) (Table 3.2b). There was no significant variability on weight of heads between genotypes; however HA89 had the highest weight (g) (25.64 ± 0.75) while HA89*5/cm90 had the lowest weight of head (24.59 ± 0.75) (Table 3.2b). There were no genotypic variations on weight of biomass (g). Nevertheless, HA89*5/HAR2 had the least weight of biomass (58.26 ± 1.13) while HA89*5/HAR3 has the highest weight of biomass (60.23 ± 1.13) (Table 3.2c). There were insignificant genotypic variability on harvest index. Despite insignificant variation, HA89 had the highest harvest index (0.183

± 0.007) while HA89*5/HAR2 had the least harvest index (0.169 ± 0.007) (Table 3.2c).

3.4.3 Allopurinol main effects

Variability due to allopurinol treatment was observed on percent disease area, number of lesions, NDSU rating score (Table 3.1a), area of head (cm^2), number of seeds, weight of seeds (g) and weight of biomass (g) (Table 3.1b) ($P<.05$). There were no significant allopurinol effects on number of days to maturity, plant height (cm), weight of stalks (g) (Table 3.1a), weight of heads (g), and harvest index (Table 3.1c) ($P>.05$). There was variability attributed to allopurinol dosage on percent disease area, number of lesions and NDSU disease rating score, where higher (100 µM) allopurinol levels gave lower values compared to lower allopurinol (50 µM) dosage and the control (0µM). The highest allopurinol dose (100 µM) had the least percent disease area (1.11 ± 0.389) while the 50µM allopurinol dose and the control (0µM) had the intermediate (3.34 ± 0.389) and the highest (7.41 ± 0.389) percent disease area (Table 3.2a). Thus, allopurinol treatment reduced percent disease area by six-fold and three-fold at 100µM and 50µM respectively when compared to the control. There was a reduction in number of lesions with an increase in allopurinol dose. There was a three-fold and two-fold reduction in number of lesions with application of allopurinol when compared with number of lesions on a control

respectively. Thus, the number of lesions for 0µM, 50µM and 100µM were 35 ± 7.62, 21 ± 7.62 and 12 ± 7.62 respectively (Table 3.2a). The NDSU disease rating scores also showed that higher allopurinol (100µM) dose had low severity ratings compared to the intermediate (50µM) and the control (0µM) (Table 3.2a). Thus, the scores were 1.257 ± 0.114 (control), 0.492 ± 0.114 (50µM) and 0.181 ± 0.114 (100µM) allopurinol doses (Table 3.2a, Fig. 3.25a, Fig. 3.25b).

Allopurinol had an effect on area of head (cm^2), where an increase in the dose of allopurinol reduced the area of the head. The control had the biggest (57.25 ± 1.30) area of head followed by the 50µM (54.25 ± 1.30) while the highest (75µM) allopurinol dose had the least (52.45 ± 1.30) area of head (Table 3.2b). However, there was no variability in area of head between the control and the 50µM allopurinol dose. Furthermore, there was no variability in area of the head between the 50µM and 100µM allopurinol doses, but there was variability on area of the head between the control and the highest allopurinol dose (Table 3.2b).

The number of seeds varied with level of allopurinol dose, thus the 50µM allopurinol dose (317 ± 8.81) and the control (305 ± 8.81) had more seeds than the 100µM allopurinol dose (282 ± 8.81) of allopurinol (Table 3.2c). However, there was no variability between the control and the 50µM treatments on number of seeds, but they differed significantly with the 100µM allopurinol dose. There

was variability on weight of seed (g) associated with allopurinol treatment. Thus, the control had higher (12.67 ± 0.31) weight of seed compared to the plants treated with 50µM (11.66 ± 0.31) and 100µM (11.15 ± 0.31) allopurinol doses. There was no variation on weight of seeds between the plants grown in allopurinol media (Table 3.2c). There was variation on weight of biomass (g) attributed to allopurinol treatment. The control produced more (60.84 ± 0.98) weight of biomass compared to the 50µM (59.03 ± 0.98) and the 100µM (58.39 ± 0.98) allopurinol doses. However, there was no variability between the control and 50µM; similarly, there was no variation between the 50µM and the 100µM allopurinol doses on weight of biomass. On the contrary, there was variability between the control and the 100µMallopurinol dose on weight of biomass (Table 3.2c).

3.4.4 Race × genotype interaction effects

There were race × genotype interaction effects on percent disease area, number of days to maturity (Table 3.1a) and number of seeds (Table 3.1b) ($P<.05$). On the contrary, there were no isolate × genotype interaction effects on number of lesions, NDSU rating score, plant height (cm), weight of stalks (g) (Table 3.1a), area of head (cm^2), weight of head (g), weight of seeds (g) weight (g) of biomass and harvest index (Table 3.1b) ($P>0.05$). There was variability on percent disease area due to race × genotype interactions. Plants inoculated with

race 336 had big disease percent area values compared with plants inoculated with race 304 race inn all the genotypes. Within the 336 race × genotype treatment, 336 × HA89*5/HAR3 (10.86 ± 0.755) and 336 × HA89*5/HAR2 (10.41 ± 0.755) had higher percent disease areas than 336 × HA89 (08.19 ± 0.755) and 336 × HA89*5/cm90 (06.63 ± 0.755) (Fig. 3.1). On the other hand when the race was 304, there was no variation on percent disease area across all the genotypes (Fig. 1). Furthermore, there was no variability in the control × genotype treatment combination on percent disease area (Fig. 3.1).

There was a significant race × genotype treatment interaction on number of days to maturity. There was lack of a general trend in in the responses due to the race × genotype interaction. Variability on days to maturity existed between HA89 and HA89*5/HAR2 genotypes when they were inoculated with race 304, but there was no variation between HA89, HA89*5/cm90 and HA89*5/HAR3. Thus, HA89 genotypes reached maturity longer than HA89*5/HAR2 when they were inoculated with race 304. Furthermore, there was no variation between HA89*5/cm90, HA89*5/HAR3 and HA89*5/HAR2 when the plants were inoculated with race 304 (Fig. 3.2). In genotypes that were inoculated with race 336, the only variation on number of days to maturity existed between HA89*cm90 and HA89*5/HAR2, where the former attained maturity earlier than the latter genotype. Nevertheless, there were no significant differences on number of days to maturity on HA89, HA89*5/cm90 and HA89*5/HAR3 when

inoculated with race 336 (Fig. 3.2). There were no variations on number of days to maturity in all the non-inoculated genotypes (Fig. 3.2). When HA89 was the genotype, 304 race inoculated plants reached maturity very late compared to the non-inoculated and 336 race inoculated plants. Thus, there was no variation in the number of days to maturity between the non-inoculated and 336 race inoculated plants when HA89 was the genotype (Fig. 3.2). The number of days in 336 race inoculated plants was lower than in 304 race inoculated and non-inoculated plants when the genotype was HA89*5/cm90. Furthermore, there was variation between the 336 race inoculated plants to the 304 race and the non-inoculated plants when genotype was HA89*5cm90 (Fig. 3.2). When HA89*5/HAR3, the plants inoculated with 336 race had less number of days to reach physiological maturity compared to the plants inoculated with 304 race and the non-inoculated plants. There was no variation on plants attaining physiological maturity between the non-inoculated and the plants inoculated with 304 race; but the non-inoculated and the 304 race inoculated plants were significantly different from the 336 race inoculated plants. There was no variation in the number of days in the 304 race and 336 race inoculated plants to the non-inoculated plants when HA89*5/HAR2 was the genotype (Fig. 3.2).

There was a significant variation in the race × genotype interaction on number of seeds. However, there was no specific trend in the number of seeds produced on the treatment combination. The number of seeds produced in the

non-inoculated and the 336 race inoculated plants was higher than in the 304 race inoculated plants when HA89 was the genotype. Furthermore, there was no variation in the number of seed produced between the non-inoculated and the 336 race inoculated plants where HA89 was the genotype (Fig. 3.3). When HA89*5/cm90 was the genotype, the 336 race inoculated plants had the highest number of seeds followed by the non-inoculated plants while the 304 race inoculated plants had the least number of seeds (Fig. 3.3). There was no variation in the number of seeds between the inoculated plants (304 race and 3336 race) and the non-inoculated plants when HA89*5/HAR3 was the genotype (Fig. 3.3). There was variation between the inoculated and the non-inoculated in number of seeds when HA89*5/HAR2 was the genotype. Thus, 336 race inoculated plants had the highest number of seeds followed by the non-inoculated plants while the 304 race inoculated plants had the least number of seeds (Fig. 3.3). In general, 336 inoculated plants had the highest number of seeds in all the genotypes. Furthermore, there was less variability in number of seeds in 336 race inoculated plants in all the genotypes except between HA89 and HA89*5/cm90 genotypes where there variation existed (Fig. 3.3). The non-inoculated plants were intermediate in the number of seeds produced in all the genotypes. There was no variation in number of seeds between HA89*5/cm90 and HA89*5/HAR3, HA89 and HA89*HA89*5/cm90 and HA89*5/HAR3 and HA89. Nevertheless, there was variation in number of seeds in non-inoculated plants between HA89 and

HA89*5/HAR3, and HA89*5/HAR3 versus HA89*5/HAR2 (Fig. 3.3). The 304 race inoculated plants had the least number of seeds than the 336 race inoculated and the non-inoculated plants except HA89*5/HAR3 genotype. There was no variation in number of seeds between HA89, HA89*5/cm90 and HA89*5/HAR2; but HA89*5/HAR3 had the highest number of seeds and was significantly from HA89, HA89*5/cm90 and HA89*5/HAR2 genotypes (Fig. 3.3).

3.4.5 Race × allopurinol interaction effects

There were significant race × allopurinol treatment interaction effects on percent disease area, number of lesions, NDSU rating score, days to maturity, weight of stalks (g) (Table 3.1a), area of head (cm^2), weight of head (g), number of seeds, weight of seeds (g), weight of biomass and harvest index (Table 3.1b) ($P<.05$). There were no significant race × allopurinol treatment interaction effects on plant height (cm) (Table 3.1a) ($P>.05$). An increase in allopurinol application resulted into a reduction in percent disease area in all the inoculated plants, except under the treatments where plants were not inoculated (Fig. 3.4). Plants inoculated with race 336 had higher percent disease area under all the allopurinol doses than plants inoculated with race 304 (Fig. 3.4).

There was a reduction in number of lesions with an increase in dose of allopurinol in the inoculated plants. The number of lesions on plants inoculated with 336 race were higher than plants inoculated with race 304 across the

allopurinol doses (Fig. 3.5, Fig. 3.25a, and Fig. 3.25b). NDSU disease severity rating score showed that plants receiving high doses of allopurinol had low scores regardless of the race used in inoculating the plants. However, plants inoculated with 336 race had higher scores at all levels of allopurinol than plants inoculated with race 304 (Fig. 3.6, Fig.3.25a, and Fig. 3.25b). There was a variation between 336 race and 304 race inoculated plants grown in a zero allopurinol media. In plants grown in 50µM allopurinol dose, there was no variation in the disease severity rating scores between the 304 race and 336 race inoculated plants. Similarly, there was no variation in the disease severity rating scores in plants grown in 100µM allopurinol dose, between the 336 race and 304 race inoculated plants (Fig. 3.6).

The presence of a significance race × allopurinol interaction effects on number of days to physiological maturity showed some trends in though not all the treatment combinations. In general the 336 race inoculated plants reached physiological maturity earlier than the 304 race inoculated and the non-inoculated plants (Fig. 3.7). In 336 race inoculated plants, the plants grown in 100µM reached physiological maturity earlier than the plants raised in the zero allopurinol dose. Furthermore, the 336 race inoculated plants grown in the 100µM media were not significantly different from the plants grown in the 50µM media on number of days to maturity. The 336 inoculated plants grown in 100µM media were significantly different from the plants grown in zero allopurinol dose

on number of days to maturity. However, the 336 inoculated plants grown in the zero dose allopurinol media were not significantly different from the plants grown in the 50µM dose media on number of days to reach physiological maturity (Fig. 3.7). There was no variability on number of days to reach physiological maturity in 304 race inoculated plants across the allopurinol dose treatments (Fig. 3.7). In non-inoculated plants, there was no variation in number of days to reach physiological maturity with an increase in dose of allopurinol (Fig. 3.7). When allopurinol was zero, there was no variation between the non-inoculated and inoculated (304 race and 336 race) plants on number of days to reach physiological maturity (Fig. 3.7). The period to reach physiological maturity was longer in 304 race inoculated plants was longer and significantly different from the non-inoculated and 336 race inoculated plants grown in a 50µM media. However, there was no variability between the non-inoculated and 336 race inoculated plants grown in a 50µM media on the duration (number of days) to reach physiological maturity (Fig. 3.7). When the plants were grown in a 100µM media, the 336 race inoculated plants reached physiological maturity early than the non-inoculated and the 304 race inoculated plants. Furthermore, there was no variation between the non-inoculated and 304 race inoculated plants on the number of days to reach physiological maturity (Fig. 3.7).

The significant race × allopurinol treatment interaction effects on weight of stalks (g) showed that the plants inoculated with 336 race had higher weight of

stalks in all the allopurinol doses than the plants inoculated with 304 race and the non-inoculated plants (Fig. 3.8). However, there was no variation between the non-inoculated and the 336 race inoculated plants under zero dosage allopurinol treatment on weight of stalks. There was no variability on weight of stalks between the 304 race inoculated and the non-inoculated plants on weight of stalks in plants grown in the 50µM and 100µM the allopurinol doses media but there was a variation between non-inoculated and the 304 race inoculated in plants grown in the zero allopurinol dose media (Fig. 3.8). There was a significant variation in area of a head (cm^2) due to race × allopurinol treatment interaction. Despite the significant interaction on the area of the head, there was no variation on plants inoculated with 304 race in all the allopurinol doses (Fig. 3.9). Plants inoculated with 336 race but grown in zero allopurinol media and the plants grown in 50µM had bigger heads than the plants grown in the 100µM dose media. Furthermore, the plants raised in the 100µM were significantly different from the plants grown in the zero allopurinol and 50µM allopurinol media. There was no variability in the area of heads in non-inoculated plants grown in the zero and 100µM allopurinol doses media. Similarly, there was no variability on the area of the head between plants grown in the 50µM and 100µM allopurinol media. Nevertheless, there was a variation in the area of head in the non-inoculated plants grown in zero and 50µM allopurinol dose media (Fig. 3.9). Plants raised in the zero media, the non-inoculated and 336 race inoculated had bigger heads than the

304 race inoculated plants that produced the lowest area of heads. Thus there was no variation in the area of heads between the non-inoculated and 336 race inoculated plants, but the two (non-inoculated and race 336 inoculated) were significantly different from the 304 race inoculated plants grown in zero allopurinol media (Fig. 3.9). The race 336 inoculated plants had bigger heads than the race 304 inoculated and non-inoculated plants grown in 50µM allopurinol media. Furthermore, there was no variation in the area of heads between the non-inoculated and the race 304 inoculated plants grown in 50µM media (Fig 3.9.). In plants grown in a 100µM allopurinol media, there was no variation in the area of head between the non-inoculated and the race 336 inoculated plants. Furthermore, there was variation in area of heads between 304 race and 336 race inoculated plants (Fig. 3.9).

There were significant differences on weight of heads (g) due to the race × allopurinol interactions. There was no variability in weight of heads between the non-inoculated and the race 336 inoculated plants grown in zero allopurinol media. Similarly, there was no variation in weight of heads between the 304 race and 336 race inoculated plants grown in zero allopurinol media. However, there was a variation in the weight of heads between the non-inoculated and the 304 race inoculated plants grown in the zero allopurinol media (Fig. 3.10). There was no variation in the weight of heads between the non-inoculated and the 336 race inoculated plants grown in 50µM allopurinol dose media. Furthermore, the

weight of heads of 336 race inoculated plants were significantly different from the 304 race inoculated plants; thus, the weight of heads was higher in 336 race inoculated plants versus the 304 race inoculated plants grown in 50µM allopurinol media. However, there was no variation in the weight of heads between the non-inoculated and 304 race inoculated plants (Fig. 3.10). The weight of heads for the inoculated (race 304 and race 336) and non-inoculated plants grown in 100µM allopurinol media, were not significantly different (Fig. 3.10). There was no variability on the 336 race inoculated plants across the allopurinol doses. In 304 race inoculated plants, there was no variation between plants grown in the zero allopurinol media and the 100µM allopurinol media. Furthermore, there was no variation in weight of heads between the 304 race inoculated plants grown in the zero allopurinol media versus the plants grown in the 50µM allopurinol media. The non-inoculated plants raised in zero allopurinol media were significantly different from the plants raised in the 50µM allopurinol media but not different from the plants grown in the 100µM allopurinol on weight of the heads (Fig. 3.10). However, there was no variation in weight of heads in plants grown in allopurinol media (Fig. 3.10).

The presence of a significant race × allopurinol treatment interaction created varied responses on number of seeds. There was variation in number of seeds between the non-inoculated and the 336 race inoculated plants grown in allopurinol free media (Fig. 3.11). The 336 race inoculated and non-inoculated

had more seeds than the 304 race inoculated plants grown in the allopurinol free media (Fig. 3.11). When the growing media was 50µM, there was no variation in the number of seeds between the non-inoculated and the 304 race inoculated plants. The 336 race inoculated plants had high seed count and significantly different from the non-inoculated and 304 race inoculated plants (Fig. 3.11). The non-inoculated plants had more seed count than the race 304 and race 336 inoculated plants grown in 100µM allopurinol media (Fig. 3.11). Furthermore, there was no variation in seed count in inoculated plants but were significantly different from the non-inoculated plants on seed count.

There was significant variation on race × allopurinol treatment interaction on weight of seeds (g). Under zero allopurinol treatment, there was no variation in seed weight between the non-inoculated and 336 race inoculated treatments but the two treatments were different from the 304 race inoculated plants. Thus, the 304 race inoculated plants gave the lowest quantity of seed weight compared to the 336 race inoculated and non-inoculated plants (Fig. 3.12). When the allopurinol level was 50 µM there were no variations in weight of seeds between the non-inoculated and the plants inoculated with 304 race, however, they were different from the plants inoculated with 336 race. The plants inoculated with 336 race had higher seed weight than the plants inoculated with 304 race and non-inoculated plants under 50µM allopurinol dosage (Fig. 3.12). When allopurinol level was 100µM, the non-inoculated plants had the highest seed weight than the

inoculated plants (336 race and 304 race). Furthermore, there was a variation between the inoculated and the non-inoculated plants (Fig. 3.12).

There was a trend on weight of biomass (g) attributed to the race × allopurinol interaction when allopurinol dosages were 50µM and 100µM than in non-allopurinol treated plants. When allopurinol was zero (control), there was no variability between the non-inoculated and the 336 race inoculated plants on weight of biomass. On the other hand, the 304 race inoculated plants were significantly different from the non-inoculated and 336 race inoculated plants on weight of biomass. Thus, the non-inoculated and 336 race inoculated plants had higher weight of biomass than the 304 race inoculated plants (Fig. 3.13). When allopurinol levels were 50µM and 100µM, the 336 race inoculated plants were significantly different from the non-inoculated and 304 race inoculated plants. Nevertheless, the non-inoculated and 304 race were not significantly different under the 50µM and 100µM allopurinol dosages. Furthermore, the 336 race inoculated plants had higher biomass weight compared to the non-inoculated and 304 race inoculated plants (Fig. 3.13).

There was a significant variation on race × allopurinol interaction on harvest index. When the allopurinol dose was zero, there was no variation between the non-inoculated and 336 race inoculated plants. Furthermore, the 304 race inoculated were significantly different from the non-inoculated and 336 race

inoculated plants. Thus, 304 race inoculated had a lower harvest index than the non-inoculated and 336 race inoculated plants (Fig. 3.14). There was no variation in harvest indices between the non-inoculated and inoculated (304 race and 336 race) plants grown in a 50µM allopurinol media (Fig. 3.14). When the allopurinol dose was 100µM, there were variations in harvest indices between the non-inoculated and inoculated (304 race and 336 race) plants. Thus, the non-inoculated plants had a higher harvest index than the inoculated plants (Fig. 3.14).

3.4.6 Genotype × allopurinol interaction effects

Variability due to genotype × allopurinol interaction effects were observed on number of seeds, weight of seeds (g) and harvest index (Table 3.1b) ($P<.05$). However, there were no significant genotype × allopurinol interaction effects on percent disease area, number of lesions, NDSU severity rating score, days to maturity, plant height (cm), weight of stalks (g) (Table 3.1a), area of head (cm^2), weight of head (g) and weight of biomass (g) (Table 3.1b) ($P>0.05$).

There was a significant genotype × allopurinol interaction on number of seeds (Table 3.1b). When HA89 was a genotype, plants grown in zero allopurinol media were significantly different from the plants grown in the 50µM and 100µM allopurinol doses media; but there was no variation on number of seeds between the allopurinol treated plants (Fig. 3.15). Thus, the plants grown in allopurinol free media had the highest seed count than the plants grown in allopurinol media.

There was no variability on number of seeds between the plants grown in an allopurinol free media and the plants grown in the 50µM and 100µM allopurinol media when HA89*5/cm90 was the genotype (Fig. 15). When HA89*5/HAR3 was the genotype, there was no variation in the number of seeds between the plants grown in zero allopurinol media to the plants grown in the 50µM and 100µM doses of the allopurinol media (Fig. 3.15). There were significant variations on number of seeds between plants grown in the allopurinol treated media and the plants grown in the allopurinol free media when HA89*5/HAR2 was the genotype (Fig. 3.15). The 50µM treated plants had the highest number of seeds followed by the plants grown in zero allopurinol media, while the plants grown in the 100µM allopurinol media had the least number of seeds (Fig. 3.15).

The significance of genotype × allopurinol interaction on weight of seeds suggests that the responses of genotypes varied with allopurinol dosage (Table 3.1b). When HA89 was the genotype, the control (zero allopurinol) the weight of seeds was higher than in allopurinol treated plants (50µM and 100µM). Furthermore, plants grown in zero allopurinol dose media were significantly different from the plants grown in the 50µM and 100µM allopurinol doses media that were not significantly different from each other on weight of seeds (Fig. 3.16). When HA89*5/cm90 was the genotype, there was no variability between the plants grown in the zero allopurinol media and the plants grown in50µM and 100µM the allopurinol media (Fig. 3.16). The weight of seeds from the plants

that grew in an allopurinol free media was higher than the plants that grew in a 50µM and 100µM allopurinol media when HA89*5/HAR3 was the genotype. Furthermore, the plants that grew in a zero allopurinol media were significantly different from the plants that grew in allopurinol media on weight of seeds. However, there was no variability between plants that grew in allopurinol media on weight of seeds. Thus, plants that grew in a zero allopurinol media had a higher weight of seed than the plants that grew in an allopurinol media (Fig. 3.16). There was variability on weight of seeds in HA89*5/HAR2 genotype between plants that grew in a 100µM dosage compared to the plants that grew in a zero allopurinol and 50µM allopurinol dose media. However, there was no variation in weight of seed between the plants that grew in the zero allopurinol media versus the plants that grew in the 50µM allopurinol dose (Fig. 3.16). The weight of seed from the plants from the 100µM allopurinol media was the lowest compared to the plants from the zero allopurinol dose and the 50µM dose media. In general the weight of seed from the zero free allopurinol media was higher than the weight of seeds from plants grown in allopurinol media. There was less variation in weight of seeds from plants that received allopurinol treatment across all the genotypes except for HA89*5/HAR2 where the plants that grew in the 100µM allopurinol media had the least weight of seeds (Fig. 3.16).

The significance of genotype × allopurinol interaction on harvest index suggests that the responses of genotypes varied with allopurinol dose (Table

3.1b). There was no variation on harvest index between the plants grown in allopurinol free media and the plants grown in allopurinol (50µM and 100µM) media when HA89 was the genotype (Fig. 3.17). Similarly, there were no variations in harvest indices between the plants grown in zero allopurinol media to the allopurinol grown plants when HA89*5/cm90 was the genotype (Fig. 3.17). There was no variation on harvest indices between plants grown in zero allopurinol media and the 100µM allopurinol dose media, they were significantly from the plants grown in the 50µM allopurinol media when the genotype was HA89*5/HAR3 (Fig. 3.17). The harvest indices in HA89*5/HAR3 plants grown in zero allopurinol media and in 100µM allopurinol were higher than in HA89*5/HAR3 plants grown in 50µM allopurinol dose. When HA89*5/HAR2 was the genotype, there was no variation in harvest indices between the plants grown in zero allopurinol media and plants grown in 50µM allopurinol media (Fig. 3.17). Furthermore, the plants grown in 100µM allopurinol dose had the lowest harvest index compared to the zero allopurinol media and the 50µM allopurinol media.

3.4.7 Race × genotype × allopurinol interaction effects

Significant variations attributed by race × genotype × allopurinol interactions were observed on percent disease area, weight of stalks (g), (Table 3.1a), weight of head (g), number of seeds, weight of seeds (g), weight of biomass

(g) and harvest index (Table 3.1b) (*P<.05*). There was no significant variation on race × genotype × allopurinol interaction effects on number of lesions, NDSU rating score, days to maturity, plant height (cm) (Table 3.1a), and area of the head (cm^2) (Table 3.1b) (*P>0.05*). The presence of significant race × genotype × allopurinol interaction on percent disease area suggests that these three factors influence the phenotype on the host. The range in percent area was from 0.002 ± 1.298 (All genotypes and allopurinol treatments not inoculated) to 20.64 ± 1.298 (0µM × 336 race × HA89*5/HR3) (Fig. 3.18). The treatment combinations that were inoculated with 336 race but grew in zero allopurinol media had higher values for percent disease area regardless of the type genotype. For instance, 0µM × 336 race × HA89*5/HAR3, 0µM × 336 race × HA89*5/HAR2 and 0µM × 336 race × HA89 treatments had high percent disease area (Fig. 3.18). There was a reduction in percent disease area with an increase in allopurinol level regardless of the genotype and race used to inoculate the plants. However, the percent disease area in 336 race inoculated plants was higher than in the 304 race inoculated plants (Fig. 3.18). Furthermore, there was no variation in percent disease area between the non-inoculated plants compared to the inoculated plants that received the highest level of allopurinol dosage regardless of the genotype (Fig. 3.18). The results suggest that allopurinol was able to suppress disease development in these treatment combinations.

Race × genotype × allopurinol interaction was significant for weight of stalks (g) trait (Table 3.1a) suggesting that weight of stalks produced is dependent on a combination of race, genotype and allopurinol dose. In general plants inoculated with 336 race had higher weight of stalks than plants inoculated with 304 race and the non-inoculated plants (Fig. 3.19). However, there was no variability on weight of stalks in HA89 genotype, inoculated with 336 race and grown in different allopurinol media. A similar trend was observed when HA89*5/cm90 was the genotype, and plants inoculated with 336 race; there was no variability in weight of stalks in plants grown different allopurinol doses (Fig. 3.19). When HA89*5/HAR3 was a genotype and were inoculated with 336 race, the plants grown in 50µM had the highest weight of stalks compared to the zero allopurinol dose and 100µM allopurinol dose. Nevertheless, there was no variation between the plants grown in the zero and 100µM allopurinol media. In HA89*5/HAR2 genotype, inoculated with 336 race, there was variability in weight of stalks between plants grown in 50µM and 100µM allopurinol media, but no variation between the zero and 50µM allopurinol on weight of stalks. Furthermore, there was no variation between the plants grown in zero and 100µM allopurinol media on weight of stalks (Fig. 3.19). In 304 race inoculated plants there was no variation on weight of stalks in all the genotypes and the media where the plants were grown (Fig. 3.19). In non-inoculated plants there were variations in weight of stalks between genotypes and allopurinol doses. When

HA89 was the genotype, plants grown in zero dose allopurinol media had the highest weight compared to the allopurinol treated plants. There was no variation in weight of stalks on the plants grown in the 50µM and 100µM allopurinol dose media (Fig. 3.19). There was variation in weight of stalks between the plants grown in 100µM allopurinol media to the zero and the 50µM allopurinol dose media in the non-inoculated HA89*5/cm90 genotype (Fig. 3.19). Thus, the plants grown in the 100µM had the highest weight of stalks compared to the zero dose and 50µM allopurinol dose treated plants. However, there was no variation between zero allopurinol dose and 50µM allopurinol dose on weight of stalks. There was no variability on weight of stalks in the non-inoculated HA89*5/HAR3 genotype plants regardless of the allopurinol dose (Fig. 3.19). The HA89*5/HAR3 genotype plants grown in zero allopurinol media had the highest weight of stalks compared to the plants grown in the allopurinol media (50µM and 100µM doses) in non-inoculated treatment (Fig. 3.19). The results in HA89*5/HAR2 suggest that allopurinol had an effect on the weight of stalks, when plants were not inoculated with a pathogen.

Race × genotype × allopurinol interaction was significant for weight of heads (g) trait (Table 3.1b) suggesting that weight of heads is dependent on a combination of race, genotype and allopurinol dose. In non-inoculated plants, there was no variation in weight of heads (g) in all the genotypes and across the allopurinol doses except for HA89*5/HAR2 genotype grown in a zero dose

allopurinol media, which had the highest weight of head and significantly different from the rest of treatment combinations (Fig. 3.20). In plants that were inoculated with race 304, only HA89 genotype grown in 100µM allopurinol dose media had the highest weight of head while the rest genotypes and allopurinol doses had similar weights of heads (Fig. 3.20). Nevertheless, HA89*5/cm90 genotype grown in 50µM had the least weight of heads compared to other treatment combinations in the 304 race inoculated plants. There was a diversity of responses in weight of heads in genotypes inoculated with 336 race grown in different allopurinol media. When HA89 was a genotype, plants grown in zero allopurinol media had the highest weight of heads and were significantly different from the plants grown in allopurinol (50µM and 100µM) media (Fig. 3.20). There was no variation in weight of heads in HA89 genotype grown in the 50µM and 100µM allopurinol doses media when they were inoculated with 336 race. Despite lack of variability between the HA89 genotype plants inoculated with 336 race grown in allopurinol media, the plants grown in the highest dose of allopurinol had the least weight of heads (Fig. 3.20). When the genotype was HA89*5/cm90, there was no variation in weight of heads in plants grown in allopurinol media and inoculated with 336 race. Furthermore, there was no variation in weight of heads between the plants grown in the zero dose and 100µM dose allopurinol dose media. Nevertheless, there was variation between plants grown in the zero dose and 50µM allopurinol dose media on weight of

heads. Thus, the plants grown in 50µM allopurinol media had the highest weight of heads followed by the 100µM allopurinol dose while the plants grown in the zero allopurinol dose had the least weight of heads (Fig. 3.20). When HA89*5/HAR3 was the genotype, there were no variations on weight of heads between the plants grown in zero allopurinol dose and the plants grown in the 50µM allopurinol dose inoculated with 336 race. However, the plants grown in the 100µM allopurinol media had the highest weight of heads and were significantly different from the plants grown in the zeros dose allopurinol and 50µM allopurinol dose media on weight of heads (Fig. 3.20). On the other hand, there were no variations in weight of heads between the HA89*5/HAR2 genotype plants grown in the zero dose allopurinol and 50µM allopurinol dose inoculated with 336 race. However, the HA89*5/HAR2 plants grown in the highest (100µM) allopurinol dose media had the least weight of heads and were significantly different from the plants grown in the zero and 50µM allopurinol media (Fig. 3.20) when they were inoculated with 336 race. Thus, there was a reduction in weight of heads with an increase in allopurinol dose in HA89*5/HAR2 genotype when they were inoculated with race 336.

The presence of a significant race × genotype × allopurinol interaction on seed number trait (Table 3.1b) suggests that number of seeds trait is dependent on a combination of race, genotype and allopurinol treatment factors. In general, there were inconsistencies on the number of seeds across the treatment

combinations, thus, there was no trend on number of seeds produced in relationship to the treatment combination. In non-inoculated HA89 genotype, there was no variation in number of seeds between plants grown in zero allopurinol media and plants grown in highest dose of allopurinol (100µM) media. Furthermore, there was no variability in non-inoculated HA89 genotype grown in zero allopurinol media and plants grown in 50µM allopurinol media on number of seeds, but there was a variation in the number of seeds between the plants grown in the 50µM and 100µM allopurinol media (Fig. 3.21). The plants (HA89 genotype) grown in the 50µM allopurinol media had the least number of seeds while the plants grown in the 100µM allopurinol media had the highest number of seeds. There was no variability in the number of seeds in non-inoculated HA89*5/cm90 genotype plants grown in different allopurinol doses (Fig. 3.21). However, HA89 plants inoculated with 336 race, there was a reduction in number of seeds with an increase in allopurinol dosage. When HA89*5/cm90 was the genotype, there was no variation in number of seeds in plants inoculated with 336 race across different levels of allopurinol media (Fig. 3.21). Nevertheless, the plants grown in the highest (100µM) allopurinol media had the least number of seeds than the plants grown in the zero allopurinol and 50µM allopurinol media. There was no variation in the number of seeds in plants grown different allopurinol doses and inoculated with 336 race when HA89*5/HAR3 was the genotype (Fig. 3.21). Despite lack of variation in the

number of seeds, the plants grown in the 50µM allopurinol dose had the least number of seeds compared to the plants grown in the zero allopurinol dose and 100µM allopurinol dose (Fig. 3.21). There was a variation in number of seeds in HA89*5/HAR2 genotype plants inoculated with 336 race grown in different allopurinol media. Thus, the plants grown in 50µM allopurinol media had the highest number of seeds than the plants grown in the zero allopurinol dose and 100µM allopurinol dose media (Fig. 3.21). Despite lack of variability between the plants grown in the zero and 100µM allopurinol doses, the plants grown in the zero allopurinol media had higher number of seeds than the plants grown in the highest allopurinol media when HA89*5/HAR2 was the genotype and inoculated with race 336.

Race × genotype × allopurinol interaction was significant for weight of seeds (g) trait (Table 3.1b) suggesting that weight of seed produced is dependent on a combination of race, genotype and allopurinol dosage. There were no consistencies in the trend of treatment combinations on weight of seeds (Fig.3.22). In non-inoculated plants, the plants grown in zero allopurinol media generally had the highest seed weights regardless of the genotype. When HA89 was the genotype, there was no variation in weight of seeds between the plants grown in zero allopurinol media and the plants grown in 100µM allopurinol media in non-inoculated treatment. Furthermore, the plants grown in the zero and 100µM allopurinol media had the highest weight of seed than the plants grown in

the 50µM allopurinol media (Fig. 3.22). In HA89*5/cm90 genotype, there was no variation on weight of seeds across the allopurinol media in non-inoculated plants. Inspite of lack of significant variation on weight of seeds, the plants grown in 50µM allopurinol dose media had the least weight of seed compared to the plants grown in the zero and highest allopurinol media (Fig. 3.22). There was variability on seed yield on HA89*5/HAR3 genotype plants grown in different allopurinol dose media in non-inoculated plants. Thus, the plants grown in 50µM allopurinol dose media had the least weight and significantly different from the plants grown in the zero and 100µM allopurinol dose media (Fig. 3.22). In non-inoculated HA89*5/HAR2 genotype, there was a reduction in seed yield with an increase in allopurinol dose. Thus, the seed yield was highest in the plants from the zero allopurinol media while the seed yield was the lowest from the plants grown in the 100µM allopurinol dose media (Fig. 3.22). There were no variations in 304 race inoculated HA89*5/cm90 genotype across all the allopurinol doses on seed yield (Fig. 3.22). Similarly, there was no variation in seed yield on HA89*5/HAR3 genotypes inoculated with 304 race grown in the zero allopurinol dose and the 50µM allopurinol dose. Furthermore, there was no variation in seed yield in HA89*5/HAR3 genotype plants inoculated with 304 race grown in the 50µM and 100µM allopurinol doses media. Nevertheless, there was seed yield variation between the HA89*5/HAR3 genotypes inoculated with 304 race grown in the zero allopurinol dose media and the plants grown in 100µM

allopurinol dose media. The results in HA89*5/HAR3 genotype inoculated with 304 race show that there was a reduction in seed yield with increase in allopurinol dose despite minimal variation between the zero dose and 50µM allopurinol dose (Fig. 3.22). When the genotype was HA89*5/HAR2, there was no variation in seed yield across all the allopurinol doses inn 304 race inoculated plants (Fig. 3.22). There was a seed yield reduction in HA89 genotype plants inoculated with 336 race with increased dose of allopurinol. Thus, the plants that grew in a zero dose allopurinol media had the highest seed yield while the plants that grew in the highest allopurinol dose media had the least seed yield (Fig. 3.22). In 336 race inoculated HA89*5/cm90 genotype, plants that grew in 50µM allopurinol dose media had the highest seed yield and significantly different from the plants that grew in the zero allopurinol and 100µM allopurinol doses media. However, there was no variation in seed yield between the HA89*5/cm90 genotype plants that grew in the zero allopurinol dose and the highest allopurinol dose media on seed yield (Fig. 3.22) when they were inoculated with 336 race. When HA89*5/HAR3 was the genotype there was no variation in seed yield between the plants that grew in the zero and 50µM allopurinol media but the former and latter treatments were significantly different from HA89*5/HAR3 genotype plants that grew in the highest allopurinol media (Fig. 3.22). Thus, the plants that grew in the highest allopurinol dose media had the least seed yield than the plants that grew in the zero and 50µM allopurinol doses media when they were inoculated

with 336 race (Fig. 3.22). Similarly, there was no variation in seed yield in HA89*5/HAR2 genotype plants inoculated with 336 race that grew in zero allopurinol and 50µM allopurinol doses media, however the former and latter treatments were significantly different from the plants that grew in the highest allopurinol dose media. The plants that grew in the highest allopurinol media had the least seed yield than the plants that grew in the zero allopurinol and 50µM allopurinol doses media in HA89*5/HAR2 genotype that were inoculated with 336 race (Fig. 3.22).

The significant variation on race × genotype × allopurinol interaction on weight of biomass (g) indicates that biomass weight is a function of race, genotype and allopurinol dose combinations (Table 3.1b). There was no specific trend in weight of biomass attributed to the race × genotype × allopurinol interaction (Fig. 3.23). In non-inoculated treatment, the HA89 genotype plants that grew in the zero allopurinol media had the highest weight of biomass than the plants that grew in the 50µM and 100µM allopurinol doses media (Fig. 3.23). There was no variation in weight of biomass in the HA89 genotype plants that grew in the allopurinol media and were not inoculated. When HA89*5/cm90 was the genotype, there was no variation in weight of the biomass across all the allopurinol dose in non-inoculated plants (Fig. 3.23). Similarly, there was no variation in the weight of biomass in non-inoculated HA89*5/HAR3 genotype plants across the allopurinol doses (Fig. 3.23). In HA89*5/HAR2 genotype, the

plants that grew in the zero allopurinol media had the highest weight of biomass than the plants that grew in the allopurinol media (50µM and 100µM doses) in non-inoculated plants. However, there was no variation in the weight of biomass in plants that grew in allopurinol media (Fig. 3.23). In 304 race inoculated HA89 genotype, there was no variation in weight of biomass across the allopurinol doses (Fig. 3.23). Despite, lack of significant variation on weight of biomass, the plants that grew in the highest allopurinol dose media had the highest weight of biomass than the plants that grew in the zero allopurinol dose and the plants that grew in the 50µM allopurinol dose media. In race 304 race inoculated HA89*5/cm90, despite lack of variation in weight of biomass across the allopurinol doses, the plants that grew in the zero allopurinol media had the highest weight of biomass than the plants that grew in the allopurinol media (Fig. 3.23). In HA89*5/HAR3 genotype inoculated with 304 race, there was a reduction in weight of biomass with an increase in allopurinol dose. Thus, the plants that grew in the zero allopurinol media had the highest weight of biomass while the plants that grew in the highest dose of allopurinol media had the least weight of biomass (Fig. 3.23). In contrast to HA89*5/HAR3 genotype, the HA89*5/HAR2 genotypes that were inoculated with 304 race, the plants that grew in the highest allopurinol dose media had the highest weight of biomass than the plants that grew in the 50µM and zero allopurinol dose media (Fig. 3.23). Furthermore, the weight of biomass from the plants that grew in the highest allopurinol dose media was significantly

different from the weight of biomass from the plants that grew in the zero allopurinol dose. However, there was no variation in weight of biomass from the plants that grew in the zero allopurinol media and the plants that grew in the 50 allopurinol media. Similarly, there was no variation in weight of biomass from plants that grew in the allopurinol media when inoculated with race 304 (Fig. 3.23). In 336 race inoculated HA89 genotype, the plants that grew in the zero allopurinol dose media had the highest biomass weight than the plants that grew in the 50µM and 100µM allopurinol dose media. However, there was no variation in weight of biomass in plants that grew in allopurinol treated media (Fig. 3.23). There was no variation in weight of biomass in 336 race inoculated HA89*5/cm90 genotype across the allopurinol doses. Despite insignificant variation on weight of biomass, the plants that grew on 50µM allopurinol dose media had the highest weight of mass than the plants that grew on zero allopurinol and 100µM allopurinol doses media (Fig. 3.23). When HA89*5/HAR3 genotype plants were inoculated with 336 race, the plants that grew in 50µM allopurinol dose media had the highest weight of biomass followed by plants that grew in 100µM allopurinol dose media; while the plants that grew in an allopurinol free media had least weight of biomass (Fig. 3.23). Despite, the weight of biomass from the plants that grew in the 50µM allopurinol dose media recording the highest biomass weight, the biomass weight was not significantly from the plants that grew in the highest allopurinol dose media. Similarly, there was significant

variation in the biomass weight from the plants that grew in the allopurinol free media and the plants that grew in the highest allopurinol dose media in HA89*5/HAR3 genotype inoculated with 336 race (Fig. 3.23). The weight of biomass in HA89*5/HAR2 genotype plants inoculated with 336 race was higher in plants that grew in the zero allopurinol media and 50µM allopurinol media than in 100µM allopurinol media (Fig. 3.23). However, there was no variation in weight of biomass in plants (HA89*5/HAR2) that grew in the zero allopurinol and 50µM allopurinol media that were inoculated with 336 race (Fig. 3.23).

The significant variation on race × genotype × allopurinol interaction on harvest index indicates that harvest index is a function of race, genotype and allopurinol dose combinations (Table 3.1b). There was no specific trend on harvest index attributed to the race × genotype × allopurinol interaction (Fig. 3.24). In non-inoculated HA89 genotype, the plants that grew in the highest allopurinol dose had the highest harvest index and were significantly different from the plants that grew in the zero and 50µM allopurinol dose media (Fig. 3.24). There was no variation in harvest index in non-inoculated HA89*5/cm90 genotype plants grown in different allopurinol dose media (Fig. 3.24). Despite lack of variability in the non-inoculated HA89*5/cm90 genotype across allopurinol media, there was a reduction in harvest index with increase in allopurinol dose. When HA89*5/HAR3 was the genotype, the plants that grew in the highest allopurinol dose media had the highest harvest index while the plants

that grew in the 50µM allopurinol media had the lowest harvest index in non-inoculated treatment. However, there was no variability in harvest indices between the HA89*5/HAR3 genotype plants that grew in the 100µM allopurinol dose media and the plants that grew in the zero allopurinol dose media (Fig. 3.24). Furthermore, there was no variation in harvest indices between HA89*5/HAR3 plants that grew in the 50µM allopurinol media and the plants that grew in the zero allopurinol media in non-inoculated treatment. There was a reduction in harvest index with an increase in allopurinol dose media in non-inoculated HA89*5/HAR2 genotype (Fig. 3.24). Thus, there was no variation in harvest indices between HA89*5/HAR2 plants that grew in the zero allopurinol media and plants that grew in the 50µM allopurinol media; nevertheless, there was variation between the plants that grew in the highest allopurinol media to the plants that grew in the zero allopurinol and 50µM allopurinol media. When HA89 genotype was inoculated with 304 race, there was no variation in harvest indices across the allopurinol doses (Fig. 3.24). Similarly, when HA89*5/cm90 was the genotype, there was no variation in harvest indices across the allopurinol doses when the genotype was inoculated with 304 race (Fig. 3.24). There was no variation in harvest indices in HA89*5/HAR3 and HA89*5/HAR2 genotype plants grown in different allopurinol doses media when they were inoculated with 304 race (Fig. 3.24). In HA89 genotype that were inoculated with 336 race, there was no variation on harvest indices in plants that grew in the zero and 50µM

allopurinol dose media; however the plants that grew in the highest allopurinol dose media had the least harvest index and was significantly different from the plants that grew in the zero and 50µM allopurinol dose media (Fig. 3.24). Thus, there was a decline in harvest indices with an increase allopurinol doses in HA89 genotype inoculated with 336 race (Fig. 3.24). Despite lack of variation on harvest index in HA89*5/cm90 genotype inoculated with 336 race across the allopurinol doses, the plants that grew in 50µM allopurinol had the highest harvest index than the plants that in the zero allopurinol and 100µM allopurinol doses media (Fig. 3.24). There were variations in harvest indices on 336 race inoculate HA89*5/HAR3 genotype grown in different doses of allopurinol media. Thus, the plants that grew in a zero allopurinol media had the highest harvest index, which was significantly different from the plants that grew in allopurinol media (Fig. 3.24). However, there was no variation in harvest indices in plants that grew in the 50µM and 100µM allopurinol media (Fig. 3.24). Despite lack of variation in harvest indices in 336 race inoculation on HA89*5/HAR2 genotype across different allopurinol doses, the plants that grew in the highest allopurinol had the least harvest index relative to the plants that grew in the zero and 50µM allopurinol media (Fig. 3.24).

3.4.8 Correlations between traits

There were very strong positive and significant correlations between weight of stalks and weight of biomass (0.9698), biomass weight and head weight (0.9317), weight of seeds and number seeds (0.9204), seed weight and biomass weight (0.9112), area of head and weight of biomass (0.9057), head weight and seed weight (0.9013) (*P<0.05*) (Table 3.3). Very strong positive and significant correlations were observed on area of head and weight of seeds (0.8987), area of head and weight of head (0.8774), number of seeds and weight of biomass (0.8768), weight of stalks and area of head (0.8559), area of head and number of seeds (0.8523) and weight of stalks and weight of seeds (0.8485) (*P<0.05*) (Table 3.3) had strong positive correlations. Furthermore, very strong positive significant (*P <0.05*) correlations were observed between weight of stalks and number of seeds (0.8467), weight of seeds and harvest index (0.8316), weight of heads and number of seeds (0.8224), weight of stalks and weight of heads (0.8149) and harvest index and number of seeds (0.7387) (Table 3.3). Significant (*P<0.05*) strong correlations were also observed between plant height and weight of stalks (0.7325), plant height and weight of biomass (0.7251). Positive moderate correlations were observed on area of head and harvest index (0.6902), plant height and number of seeds (0.6803), plant height and area of head (0.6736) and plant height and weight of seeds (0.6676) (Table 3.3). Moderate positive significant (*P<0.05*) correlations were observed between weight of heads and harvest index (0.6465), plant height and weight of heads (0.6318), weight of

biomass and harvest index (0.5913), percent disease area and NDSU rating score (0.5913) and NDSU rating score and number of lesions (0.5800) (Table 3.3). There were weak but positive significant correlations ($P < 0.05$) between percent disease area and number of lesions (0.5438), weight of stalks and harvest index (0.5093) (Table 3.3).

There were weak negative significant correlations between number of lesions and weight of biomass (-0.1931), number of lesions and weight of heads (-0.1874), number of lesions and area of heads (-0.1825) and number of lesions and weight of stalks (-0.1823) ($P<0.05$) (Table 3.3). Furthermore, weak negative but significant ($P< 0.05$) correlations were observed between number of lesions and weight of seeds (-0.1744), number of lesions and number of seeds (-0.1542), number of lesions and plant height (-0.1482) (Table 3.3).

3.5 Discussion

The results show that there were significant differences on percent disease area, number of lesions and NDSU disease rating score attributed to race. The disease area under 336 race was higher compared to the one under 304 race and control. The disease area under 336 race was 903 times more than the control and three times larger than on the leaf samples inoculated with 304 race. The results suggest that race 336 was more virulent compared to 304 race on causing

sunflower rust. The number of lesions on plants inoculated was higher on 336 race compared to 304 race and control treatments. There was an eight-fold and 1.5 fold differences between 336 race inoculated versus control, and 336 race versus 304 race on number of lesions on genotypes under experimentation. NDSU disease rating scores also showed that plants inoculated with 336 race had a higher score compared to 304 race and the control (not inoculated). The high percent disease area, lesion number and NDSU rating score in 336 race over 304 race suggests that 336 race was very virulent despite both isolates having a virulence classification (Gulya & Markell, 2009). Friskop et al. (2015), reported that 336 race has many differential lines unlike 304 race used in validating the level of resistance in cultivar development. This suggests that the level of virulence in 336 race is greater than in 304 race, which supports the findings observed in the study.

There was no genotypic variability on percent disease area, number of lesions and NDSU disease rating score suggesting that there was no added advantage in the lines possessing the resistance against the pathogen. Thus, the pathogens were able to induce a disease in resistant lines similar to the susceptible line. There was variability on percent disease area, lesion number and NDSU disease rating score attributed to allopurinol treatment. The control (0µM) had higher values for percent disease area, number of lesions and NDSU rating scores compared to the 50µM and 100µM treatments. The results suggest that

allopurinol effectively reduced disease progression in sunflower. The results in the study agrees with observations reported in the use of allopurinol chemical in wheat under compatible reaction disease study against leaf rust; where Ádám et al. (2000) reported that there was a reduction in disease symptoms and amount of urediospores in plants under high allopurinol dosage compared to the control plants.

The presence race × genotype interaction on present disease area suggests that there percent disease area trait affected by the genotype and race used in inoculating the plants. From the study, genotypes inoculated with 336 race produced a higher percent area compared to the genotypes inoculated with 304 race. The suggested hypothesis is that 336 race is more virulent than 304 race since it has many differential lines used in screening against sunflower (Friskop et al., 2015). Lack of variability of 304 race across the genotypes suggests that the resistant genes were able to give similar responses to race 304 in disease development. There was no variability on number of lesions and NDSU rating score traits in a genotype × race interaction. This suggests that the susceptible line performed equally the same as the lines with resistance genes.

There was a race × allopurinol interaction on percent disease area, number of lesions and NDSU disease rating score. This suggests that there was lack of consistency in the performance of genotypes in relationship with race on percent

disease area, number of lesions and NDSU scores. The results show that percent disease area, the number of lesions and NDSU scoring rate showed a progressive reduction in values with a corresponding increase in the dosage of allopurinol. The results suggests that higher levels of allopurinol were able to slow down disease development in inoculated plants. Furthermore, the results from the study agrees with observations reported by Martin et al., (1984) on isolate × fungicide and isolate × fungicide concentration pathogen sensitivity on controlling *Rhizoctonia solani* and *Rhizoctonia*-like Fungi in-vitro; they observed that there was an interaction between isolate × concentrations on the sensitivity of the pathogen. The study also shows that the percent disease area, number of lesions and NDSU rating score were higher genotypes inoculated with 336 race than in 304 race inoculated plants. The results suggest that 336 race was more virulent than 304 race. Thus, higher dosages of allopurinol were able to control pathogen growth compared to the lower and zero dosage. The results from the study agree with observations reported by Marte & Montalbini (1999) on reduction in symptoms of pathogen and diseases development in plants treated with allopurinol when inoculated with a pathogen.

There were no significant genotype × allopurinol interactions on percent disease area, number of lesions and NDSU rating scores. Lack of variability suggest that all the genotypes performed equally under all the allopurinol levels.

Lack of genotype × allopurinol level on number of lesions and NDSU rating score indicate that the treatment combinations performed equally.

At higher order level combinations, there was no race × genotype × allopurinol interaction on number of lesions and NDSU rating score. This suggests that the treatment combinations performed the same regardless of the genotype, race and allopurinol level. On the contrary, there was a race × genotype × allopurinol interaction on percent disease area, suggesting that the variability on percent disease area was not consistent amongst the treatment combinations. The treatment combinations with 336 race had higher percent disease area values regardless of the genotype and allopurinol level compared to 304 race. This suggests that 336 race was more virulent than 304 race. The hypothesis is that 336 race is more virulence than 304 race as it has been reported to have more differential lines than 304 race (Friskop et al. 2015). Plants with that were not inoculated registered the least percent disease area since they were a control. There was a reduction in percent disease area with an increase in allopurinol level in all the genotypes as well as the races in inoculated plants. The trend suggests that allopurinol was effective in reducing the disease development in all the genotypes as well as reducing the pathogenicity of the 304 and 336 races.

There were significant differences on days to maturity, weight of stalks and area of the head attributed to race. The presence of significant race effects on days to maturity, weight of stalks and area of the head indicate that race affected some physiological responses in the genotypes hence creating variability on number of days to maturity, weight of stalks and area of the head. On the contrary, there was no variability on plant height and weight of the head attributed to race suggesting that inoculated and non-inoculated plants performed equally on plant height and weight of head traits. There was no variation between the non-inoculated and the 304 race inoculated plants on number of days to maturity. On the other hand, there was variability on number of days between 336race inoculated plants versus the 304 race inoculated and non-inoculated plants. The 336 race is more virulent than 304 race as a result as a result it induced early onset to maturity compared to the control and 304 race. The results agree with observations reported on Arabidopsis plants where very virulent, high dosage and diverse pathogens induced early onset of flowering and maturity compared to the less virulent, low dose and less diverse pathogen community (Korves & Bergelson, 2003). Furthermore, other studies also reported that changes induced by pathogens are similar to the ones induced by abiotic stresses that promote production of salicylic acid, jasmonic acid and ethylene (Beers & McDowell, 2001; Wang, et al. 2002). One of the evolutionary models also suggested that when plants are inflicted with pathogens, usually they rapidly transition from

vegetative to reproductive phases so that they flower and produce some seeds for the next generation (Agnew, et al. 2002). There was a variation on weight of stalks attributed to race, where plants inoculated with race 336 had higher weight of stalks than race 304 and the control. The plants inoculated with race 304 had the least weight of stalks relative to the control (0), the suggested hypothesis is that the pathogen disrupted the normal growth and development hence affecting the growth parameters and weight of stalks being one of the traits (PAZARLAR, et al. 2013). The area of heads in plants under control (non-inoculated) and 336 race inoculated was larger than to plants inoculated with 304 race. The area of heads under non-inoculated plants was larger than the plants inoculated with 304 race the hypothesis being that the plants in the former treatment grew in the pathogen stress free environment unlike the plants that were inoculated with 304 race. The performance of the inoculated plants was low due to the stress induced by the pathogen as was also reported in rice grown under high fungi blast condition, which had low yield and yield components responses due to blast disease pressure (Koutroubasi, et al. 2009). Plants inoculated with 336 race on the other hand produced the largest area of the head, despite race 336 being a more virulent strain than 304 race. The explanation for this observation is lack of further reinfection and spread of the pathogen, since the greenhouse environment did not simulate natural environment where the pathogen rapidly multiply on hosts causing a reduction in yield and yield component parameters (Friskop, et al.

2011). Lack variability on plant height by race suggests that races did not have an effect on the height of the plants. The results on plant height contradict the observations from PAZARLAR, et al. (2013) where they reported a reduction in growth parameters, where plant height was one of the traits. The other possible suggestion for non-significance is that plants were raised in a greenhouse under controlled environment where the spread of inoculum was restricted to the leaves that were inoculated. Lack of variability on weight of head attributed to race suggests that the inoculated plants performed the same as the non-inoculated plants. The suggested hypothesis is lack of subsequent multiplication, recombination and reinfection of the pathogens in their respective treatments during the lifecycle of the plants in the greenhouse.

Significant genetic variability was observed on plant height and neither on number of days to maturity, weight of stalks, area of head and weight of head. Lack variability on days to maturity, weight of stalks, area of head, and weight of heads suggests that all genotypes performed equally on the traits. The variability on plant height may be attributed to the effect of the introgressed resistant genes in the susceptible line, since some genes may have a confounding effect on the background (Wu, et al. 2013; Visscher, et al. 2015).

There were no significant variations attributed to allopurinol on number of days to maturity, plant height, weight of stalks and weight of head except for area

of the head where allopurinol had an effect. This suggests that there was no added advantage in applying the allopurinol chemical to the plants as the results show that the control performed the same as the treated plants. There was variability on area of head attributed to allopurinol treatment. Thus, an increase in allopurinol dose lead to a reduction in the area of the head, suggesting the implications of inhibitory effects of allopurinol on xanthine oxidase. The effects observed in the study agree with the observations reported on silenced xanthine dehydrogenase in Arabidopsis genotypes on the floral organs compared to the wildtype (Nakagawa, et al. 2007). Therefore, higher dose of allopurinol implies that it effectively inhibit xanthine oxidase in the process affecting the photosynthetic process, ultimately reducing the quantity of photosynthates. Silencing or inhibiting xanthine oxidase affects yield and yield components traits (Nakagawa, et al. 2007. Lack of allopurinol treatment effect on weight of heads trait, suggests that weight of heads were not affected by xanthine oxidase inhibition. However, these results contradict to the observations reported by (Nakagawa, et al. 2007) on RNAi xanthine dehydrogenase silenced Arabidopsis, where the silenced genotypes had reduced reproductive organs unlike the wildtype.

There was a significant genotype × race on number of days to maturity, but no genotype × race on plant height, weight of stalks, area of head and weight of heads. The treatments inoculated with race 336 attained physiological maturity

earlier than the control and the ones inoculated with race 304. The hypothesis suggested is that race 336 is a virulent isolate compared to race 304 as a result it induced an enormous stress to the plants forcing the plants to undergo rapid transition from vegetative to reproductive phase. The rapid transition is an adaptation mechanism within the plants that enable the plants to produce seed for the next generation if stressed with abiotic and biotic factors (Agnew, et al. 2002). Lack of variability of genotype × isolate on plant height, weight of stalks, area of head and weight of heads indicate that all the genotypes performed the same under pathogen inoculated and non-inoculated environment.

There were significant race × allopurinol treatment interactions on number of days to maturity, weight of stalks, area of head and weight of head but no variability on race × allopurinol on plant height. Lack of race × allopurinol on plant height suggests that inoculated plants performed the same as non-inoculated at varying allopurinol doses. Thus, plant height was not affected by race or allopurinol chemical. The suggested explanation is that there was no further reinfection or spread of the pathogens to the plants since the experiment was implemented under greenhouse conditions unlike under natural infestation conditions. Under natural conditions, the environmental factors may promote reinfection process of the pathogen to the host (Friskop, et al. 2011). On number of days to maturity, the presence of significance on race × allopurinol interaction suggests that variability in number of days to maturity is dependent on the race

and allopurinol treatment combinations. The allopurinol treatments with 336 race generally had an earlier onset to maturity unlike the ones with race 304 and non-inoculated treatments, suggesting that the stress induced by the more virulent 336 race made the plants to attain maturity earlier than the rest of the treatments. This is one of the mechanisms that plants use to in speeding up reproduction phase so that they produce seed for subsequent generations when stressed (Agnew, et al. 2002). Furthermore, within the 336 race, the 336 race × 0µM treatment combination attained physiological maturity later than the rest of the treatments because allopurinol chemical induces early onset leaf senescence (Nakagawa, et al. 2007), which is another stress on the plants. Thus extra stress induced the plants to attain early maturity than the zero treatment in the 336 × allopurinol treatment combination. On the other hand, the case was different with 304 race, where there was minimal variability across the allopurinol dose, suggesting that the race was not very virulent as a result the effects of the race and allopurinol were not high. There was a significant race × allopurinol on weight of stalks interaction, implying that weight of stalks varies with the treatment combinations, thus there was no consistency. Under the non-inoculated × allopurinol treatments, the zero allopurinol treatment had higher weight of stalks relative the allopurinol treated plants. The results agree with study on xanthine dehydrogenase silenced genotypes of Arabidopsis had lower biomass compared to the wild type (Nakagawa, et al. 2007), implicating allopurinol on weight of stalks in the study.

Lack of genotype × allopurinol interaction on number of days to maturity, plant height, weight of stalks and area of head traits suggest that the all the genotypes performed the same on all levels of allopurinol. Thus, xanthine oxidase inhibitory effects did not have an effect on weight of stalks, plant height, days to maturity and area of head in all the genotypes despite the having a role in reducing the percent disease area and number of lesions. A higher-level interaction showed that race × genotype × allopurinol interaction was significant on weight of stalks, area of head and weight of head but not significance of number of days to maturity and plant height. The weight of stalks is a function of race, genotype and allopurinol treatment combinations. Treatments inoculated with 336 race had relatively higher weights regardless of the allopurinol treatment dose and genotype compared to the treatments inoculated with 304 race. The non-inoculated treatments were evenly distributed on weights of stalks. Under ideal situations, weight of stalks from virulent strains was supposed to be lower than a mild strain, but in the study, the more virulent strain had higher weight than a less virulent strain. The probable explanation is that there was no further reinfection and spread of the pathogen because the environment in the greenhouse did not promote reinfection process of the pathogens on the hosts. Unlike under natural conditions, despite environmental conditions vary considerably but most of the times the conditions favor multiplication, recombination and reinfection of the pathogen (Friskop, et al. 2011) during the growth and reproductive phase of the

host. These conditions were not ideal under greenhouse, as a result, the pathogens did not have the opportunity to cause damage as they would have done under natural conditions on a host.

There were significant differences on weight of seeds, number of seeds, number of seeds and harvest index attributed to race. The presences of variability on number of seeds attributed to race indicate that inoculation has an effect on seed number produced on sunflower plants. The control treatment had more number of seeds than 304 inoculated plants, illustrating the effect of a pathogen on seed production. The observations in the study agree with the results reported on wheat, where they reported a reduction in yield trait components in winter cultivars that were inoculated than in the non-inoculated winter cultivars against stripe disease (Quincke et al. 2012). On the other hand, plants inoculated with 336 race produced the largest amount of seed than the control and 304 race inoculated plants. The results contradict the observations reported by many authors; thus, pathogen-inoculated plants ought to produce less seeds compared to non-inoculated plants. The hypothesis is that the pathogen did not multiply, recombine and subsequently cause further reinfection in the plants since the greenhouse conditions were not similar to the field natural conditions where reinfection occur. Plants in the greenhouse were able to grow without pathogens inducing disease development during the later stages of the plant's lifecycle hence minimizing the effects of the pathogen on number of seeds.

Pathogens usually affect the normal physiological processes in hosts ultimately affecting the performance of hosts. The results show that the weight of seeds in the non-inoculated seeds were similar to the ones inoculated with 336 race. The plants that inoculated with 304 race gave the least weight of seed compared to the non-inoculated and inoculated with 336 race. The results contradict to observations reported by other scholars on effect of pathogen infestation on seed yield where they reported yield losses on inoculated plants (Carlson, 1987 and Gascuel et al. 2015). The probable explanation hinges on lack of reinfection of the pathogen during the adult and reproductive phases of the plants that have been reported to reduce yield of seed (Carlson, 1987) under greenhouse facilities. Under field conditions, when environmental conditions are favorable for pathogen multiplication, studies have reported that rust pathogen causes disease epidemics that reduces yields to sunflower.

The variation attributed to race showed that plants inoculated with 336 race had the highest weight biomass compared to the plants inoculated with 304 race and the control. The control gave relatively higher biomass weight than the ones inoculated with 304 race hence being in agreement with other authors who reported on impact of pathogen stress over biomass production (Lledó et al. 2015). On the other hand, plants treated with 336 race gave higher biomass than the control and the plants inoculated with 304 race. This arose due to the inability

of the pathogens to multiply and infect the host during adult stages of the host, which is more devastating as reported by Carlson (1987).

Genotypic variation was observed on weight of seeds and number of seeds but not on weight of biomass. Lack of genetic variability on weight of biomass suggests that the introgressed resistant genes did not have an effect on the identical background. However, the presence of variation on weight of seeds number of seeds suggests that resistant genes had an effect on the common background. HA89*5/HAR3 had the highest number of seeds compared to the rest of the genotypes, while HA89*5/HAR2 had the least number of seeds. The number of seeds in HA89 were slightly more than in HA89*5/HAR2 suggesting that the HAR2 gene may have a negative effect on the seed number trait. The presence of genotypic variation on seed weight suggests that the resistant genes introgressed in the susceptible line had an effect on seed weight trait. Nevertheless, HAR2 gene reduced the seed weight in the susceptible line since it had the least weight compared to other resistant genes introgressed in the susceptible lines suggesting the cost of resistant gene on yield as reported in a study on genetic modified wheat lines (Zeller, et al. 2012).

There was variability on weight of seeds, number of seeds and weight of biomass attributed to allopurinol dosage. An increase in allopurinol dosage lead to a reduction in the number of seeds, suggesting the implications of inhibitory

effects of allopurinol on xanthine oxidase. The effects observed in the study agree with the observations reported on silenced xanthine dehydrogenase Arabidopsis genotypes on the floral organs compared to the wildtype (Nakagawa, et al. 2007). High dosage of allopurinol inhibit xanthine oxidase in the process affecting the photosynthetic machinery, ultimately reducing the quantity of photosynthates that go to the developing seeds (Nakagawa, et al. 2007). In the study, high doses of Allopurinol affected the number of seeds produced compared to the zero dosage.

There was variation attributed to allopurinol treatment on seed weight. Higher dosage of allopurinol gave the least seed weight than the lower dosage and the zero allopurinol dosage. The high dosage interfered with the activities of xanthine oxidase in a similar fashion reported by Nakagawa et al. (2007) on RNAi xanthine dehydrogenase silenced Arabidopsis genotypes. The partially and completely silenced genotypes had reduced reproductive organs, reduced seed weights and numbers, which are similar to the results reported in this study. Suggesting that high allopurinol dosage has a penalty cost on yield traits.

There was a variation on weight of biomass attributed to allopurinol dosage. The results show that there was a penalty cost on weight of biomass with an increase in allopurinol dosage, suggesting that inhibiting the activity of xanthine oxidase has an effect on biomass production in the host. The results in

the study agree with observations reported by Nakagawa et al. (2007) on fresh weight of biomass on RNAi xanthine dehydrogenase silenced genotypes of Arabidopsis whose weights were low compared to the wildtype. The suggested hypothesis was that oxidase plays a role in synthesis of chlorophyll so any interference with chlorophyll synthesis automatically affects photosynthetic activities.

Variability attributed to race × genotype was observed on number of seeds only; there was no race × genotype variability on weight of seeds and weight of biomass. Lack of variability on race × genotype suggests that all the treatment combinations performed equally on weight of biomass and weight of seeds. One of the suggested reasons may be absence of pathogen multiplication and reinfection in the host during the late stages of the lifecycle may have contributed to the inoculated plants behave as the non-inoculated plants across all the genotypes. There was no consistency on seed number attributed to the race × genotype interaction. However, the genotypes inoculated with 336 race produced more seeds than the ones inoculated with 304 race while the non-inoculated were intermediate in number of seed trait. This type of response may suggest that 304 race had an enormous effect compared to 336 race on number of seeds. The response of HA89*5/HAR3 genotype was also less variable across races compared to other genotypes with the seed number trait being intermediate to high producing. However, the lack of reinfection of the pathogen to the host

during the reproductive stage of the hosts may have confounding effects on seed number trait.

There were race × allopurinol interaction on weight of seeds, number of seeds and weight of biomass traits, suggesting that lack of consistency on the responses due to the treatment combinations. Inconsistences on seed number were also observed due to significant race × allopurinol interaction. There were contradictory responses on race × allopurinol interactions on number of seeds. In plants that were inoculated with 336 race, there was a net benefit with allopurinol application up to 50µM level, beyond this dosage there was a reduction in number of seeds. The results suggests that there was a penalty cost associated with increase in allopurinol dosage on the reproductive development of the plants inoculated with 336 race. The observation suggests the negative effects of higher dosage of allopurinol on reproductive organs of plants (Nakagawa, et al. 2007). There was no variability on the number of seeds in plants inoculated with 304 race with an increase in allopurinol level, suggesting that there was no net benefit with allopurinol application on allopurinol in plants inoculated with 304 race. The probable reason behind this observation is that there was no pathogen in the host during the reproductive stage that would have induced a disease in the host especially in the control and intermediate allopurinol dosage. The case was different with the plants that were not inoculated, where the intermediate allopurinol dosage had the least number of seeds compared to the zero and high

allopurinol dosage. The results from the study especially on the high allopurinol dosage, agrees with the observations reported by Nakagawa et al. (2007) on effects of RNAi xanthine dehydrogenase silenced genotypes of Arabidopsis that had reduced number of seeds compared to the wild types. The suggested hypothesis is that inactivating xanthine oxidase negatively affects the photosynthetic machinery that leads to reduced photosynthates production ultimately reducing grain filling.

There were inconsistencies on weight of seeds attributed to the significance of the race × allopurinol interaction. At higher level of allopurinol dosage there was a reduction in seed weight with inoculation treatment; thus the non-inoculated plants had higher seed weight compared to the inoculated plants. The results suggests the negative effects of allopurinol and also the effect of inoculation on seed yield. However, the non-inoculated plants raised under zero allopurinol medium as well as the 336 race inoculated plants raised in zero allopurinol and intermediate allopurinol dosage had higher seed weights compared to plants grown in the highest allopurinol dosage. The results in the study are in agreement with observations reported by Nakagawa et al. (2007) on use of partially and completely xanthine dehydrogenase enzyme on seed yield. He reported that the silenced Arabidopsis genotypes had reduced reproductive organs and seed yield compared to the wildtype. The results suggest that there is a penalty cost associated with an interference on xanthine oxidase enzyme.

The results on effect of race × allopurinol interaction on weight of biomass show that the interaction was significant, creating inconsistencies on weight of biomass over the two factors. There was no effect of plants inoculated with 304 race grown in different allopurinol media on weight of biomass. On the other hand, there was a reduction in weight of biomass in the non-inoculated plants when grown in allopurinol media, suggesting the effect of allopurinol on biomass production. Furthermore, there was a penalty cost with increase in allopurinol dosage on plants inoculated with 336 race, where there was a reduction in weight of biomass at a higher dosage of allopurinol compared to the intermediate and the zero dosage. The absence of the pathogen during the adult and reproductive stages of the plants may have affected the outcome of the results on biomass weight. Under field conditions when the environmental conditions favors further multiplication and reinfection of the rust pathogen to sunflower hosts, the biological and yield losses occur. In case of our study, there was no further opportunity for the pathogen to multiply and reinfect the host, as a result the plants grew in a disease free environment hence having minimal variations on biomass attributed to pathogen.

Variations attributed to genotype × allopurinol interactions were observed on weight of seeds and number of seeds but not on weight of biomass. Lack of variability on genotype × allopurinol on weight of biomass suggests that the genotypes performed equally on all levels of allopurinol; thus there was not net

benefit of allopurinol treatment on biomass production. A trend was observed between allopurinol level and genotype interaction on weight of seeds in some treatment combinations. The trend showed that the control (zero) had higher seed weights than the allopurinol treated plants in HA89, HA89*5/HAR3 and HA89*5/HAR2 while in HA89*5/cm90 there was no benefit in applying allopurinol on weight of seed. The results show the negative effects of suppressing the activity of xanthine oxidase on seed yield. Effects of suppression of xanthine oxidase on seed yield were reported in xanthine dehydrogenase Arabidopsis silenced lines that had less seed weight and number compared to the wildtype (Nakagawa, et al. 2007). A similar trend was observed on number of seed trait, where plants that grew in a zero allopurinol media had higher seed number unlike in allopurinol media across the genotypes except in HA89*5/HAR2. In HA89*5/HAR2, the plants that grew in a 50µM had the highest number of seeds while the plants that grew in the 100µM had the least number of seeds. The results are in agreement with observations reported by Nakagawa et al. (2007) on Arabidopsis seed yield in genotypes whose xanthine dehydrogenase had been silenced compared to the wildtype. The wild type had higher seed number compared to the former, since in the wildtype the xanthine dehydrogenase enzyme had not been compromised. The results demonstrate that interference with xanthine oxidase by applying allopurinol have a cost on seed number.

Higher-level race × genotype × allopurinol interactions were observed on weight of seeds, number of seeds and weight of biomass traits. Presence of significant interaction suggests the responses on weight of seeds varied according to specific treatment combinations. There was no consistency on the treatment combinations on responses to weight of seeds, number of seeds and weight of biomass. Some trends were observed on weight of seeds due to race × genotype and allopurinol level on weight of seeds but the responses were not across all the treatment combinations. For instance, in some treatment combinations, there was a reduction in weight of seeds as the level of allopurinol was increased suggesting the effects of allopurinol though there were no variations on seed weight between the non-inoculated versus the inoculated treatments having the same genotype and allopurinol combinations. In other treatment combinations there were no variations in weight of seed regardless of the treatments suggesting that all the treatments performed the same, hence showing no any net benefit on use of chemical in increasing weight of seed. One of the contributing factors that lead to these inconsistencies was absence of the pathogens during the reproductive stage of the host. Lack of disease presence during the reproductive stage in all the treatments had a confounding effect on the genotypes and allopurinol. Under field conditions, when rust infection occurs during the reproductive stage, studies have indicated that they affect yield losses. In case of our study, there was no

disease presence during the reproductive phase hence all the plants growing under disease free environment.

There was no specific pattern in the number of seeds attributed to the significant race × genotype × allopurinol interaction. Lack of trend in the higher order interaction on number of seeds trait illustrate that treatment combinations affected differently the number of seeds. Furthermore, lack of variation between the non-inoculated and inoculated treatment combinations on number of seeds may be alluded to lack of pathogen presence during the reproductive phase of the genotypes across the treatments. Studies have reported the negative effects of rust disease on yield if not controlled during the reproductive phase of the plants. Absence of the rust disease during this stage may have contributed to the results obtained in the study. In other treatment combinations, there was a trend where an increase in allopurinol dosage resulted in a reduction in number of seeds. This trend in results suggests that higher level of allopurinol reduced the activity of xanthine oxidase, which plays a vital role in chlorophyll synthesis. The disruptions in chlorophyll activity resulted into a reduction in photosynthates production ultimately reducing the quantity sent for grain filling. The observation are in agreement with the results reported on xanthine dehydrogenase silenced genotypes in Arabidopsis, where the partially silenced and completely silenced genotypes had few seeds, high number sterile and infertile seeds compared to the wildtype (Nakagawa, et al. 2007).

The presence of significant race × genotype × allopurinol interaction on weight of biomass resulted in lack of a general trend in the weight of biomass across the treatment combinations. In some of the treatment combinations the weight of the biomass was higher in the zero allopurinol dosage than the allopurinol treated treatments regardless of the genotype. This type of trend may suggest the effect of the allopurinol in disrupting the photosynthetic machinery ultimately affecting biomass production than in the zero allopurinol treated plants. Nakagawa et al. (2007), reported that there was a reduction in biomass in Arabidopsis genotypes that were partially and completely silenced for xanthine dehydrogenase enzyme. The observation reported by Nakagawa et al (2007), agrees with the observations in our study on the trends where an increase in allopurinol level resulted in the reduction of weight of biomass. However, in other treatment combinations the allopurinol treated plants had higher weight of biomass than the zero allopurinol grown plants. Furthermore, other treatments combinations showed any variation between the plants grown in allopurinol media and zero allopurinol grown plants. This trend may suggest that there was no any benefit growing plants in allopurinol for biomass production. The results also show that there was no trend on the effect inoculated versus the non-inoculated plants, nor the variation between the 304 and 336 races inoculated plants on weight of biomass. Lack of pathogen presence inducing rust disease during the adult and reproductive phases of the plants may have confounding

effects on the weight of the biomass. Studies have reported the effects of the disease on biological and economical yields in many crops. In case of our study, there was no pathogen presence during the adult and reproductive phase, suggesting that the effects of the pathogen on the host did not exist to induce variation.

3.6 Conclusions

The results from the study revealed some important information on the role of allopurinol chemical in suppressing sunflower rust disease in sunflower near isogenic lines with HAR2, HAR3 and cm90 resistant genes introgressed in a susceptible line, HA89. The results show that higher levels of allopurinol chemical were able to effectively reduce the percent disease area, number of lesions and even the sunflower rating scores showed that the reduction in severity score of the disease. Plants under the control (zero allopurinol) regime showed high values for percent disease area, high number of lesions and high scores of disease severity scores. The presence of genotype × race interaction revealed that 336 race was more virulent than 304 race since the former had higher values of percentage area in all the genotypes compared to the latter that had lower values of percentage area.

On yield and yield components traits, the results from the study show that there was a penalty cost associated with higher allopurinol treatment. A high dose

of allopurinol effectively reduced the activity of xanthine oxidase but the consequences were felt on number of seed, weight of seed and other traits associated with reproductive organs. Thus, the number of seeds, weight of seeds and weight of biomass were reduced at high allopurinol dosage. Effects of isolate were contradictory on traits quantified at reproductive stages of the study since there was no multiplication and subsequent reinfection of the pathogen during the later stages of the growth and reproductive phase of the host. In future, there will be a need to reconsider applying inoculation studies at reproductive phase of the hosts so that they simulate the natural conditions that associate with pathogen infecting the plants when they are in reproductive phase.

In future, there will be a need to include wet chemistry in the study; this information will assist in quantifying changes in xanthine oxidase at each level. The information will assist in tying the loose ends that exist on the study on the physiological responses quantified in the study.

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Table 3.1a: Analysis of variance for percent disease area, number of lesions, NDSU rating score, days to maturity, plant height and weight of stalks

Effect	Percent disease area (%)	Number of lesions	NDSU rating score	Days to maturity	Plant height (cm)	Weight of stalks (g)
Race	*P<.0001*	*P<.0001*	*P<.0001*	*P<.0001*	*0.1972*	*P<.0001*
Genotype	*0.0555*	*0.7157*	*0.5938*	*0.1747*	*P<.0001*	*0.4706*
Allopurinol	*P<.0001*	*P<.0001*	*P<.0001*	*0.5187*	*0.4162*	*0.0668*
Race × genotype	*0.0383*	*0.8647*	*0.4401*	*0.0292*	*0.4545*	*0.4592*
Race × allo.	*P<.0001*	*P<.0001*	*P<.0001*	*0.0413*	*0.0886*	*0.0222*
Genotype × allo.	*0.0485*	*0.9217*	*0.8724*	*0.5409*	*0.6605*	*0.0638*
Race × gen × allo	*0.0140*	*0.0565*	*0.4762*	*0.1786*	*0.0938*	*0.0001*

Where gen = genotype and allo = allopurinol

Table 3.1b: Analysis of variance for area of head, weight of head, number of seeds, weight of seeds, weight of biomass and harvest index

Effect	Area of head (cm^2)	Weight of head (g)	Number of seeds	Weight of seeds (g)	Weight of biomass (g)	Harvest index
Race	*P<.0001*	*0.1528*	*P<.0001*	*P<.0001*	*P<.0001*	*0.0027*
Genotype	*0.2223*	*0.5797*	*P<.0001*	*0.0030*	*0.3384*	*0.2038*
Allopurinol	*0.0011*	*0.0860*	*0.0003*	*P<.0001*	*0.0351*	*0.2199*
Race × genotype	*0.3660*	*0.0617*	*0.0362*	*0.1501*	*0.4817*	*0.0774*
Race × allo.	*0.0173*	*0.0387*	*P<.0001*	*P<.0001*	*0.0018*	*0.0079*
Genotype × allo.	*0.1852*	*0.0977*	*0.0040*	*0.0126*	*0.1306*	*0.0041*
Race × gen × allo	*0.0052*	*P<.0001*	*P<.0001*	*0.0001*	*P<.0001*	*0.0120*

Where gen = genotype and allo = allopurinol

Table 3.2a: Percent disease area, number of lesions, NDSU rating scores and days to maturity associated with race, genotype and allopurinol main effects

Factor	Percent disease area	Number of lesions	NDSU rating score	Days to maturity
Race				
Control (0)	0.01 ± 0.39c	5 ± 7.62c	0.005 ± 0.1c	123 ± 0.67a
304	2.83 ± 0.39b	26 ± 7.62b	0.819 ± 0.1b	124 ± 0.67a
336	9.03 ± 0.39a	38 ± 7.62a	1.106 ± 0.1a	121 ± 0.67b
Genotype				
HA89	3.63 ± 0.45a	22 ± 7.67a	0.639 ± 0.1a	124 ± 1.21a
HA89*5/cm90	3.16 ± 0.45a	24 ± 7.67a	0.714 ± 0.1a	122 ± 1.21a
HA89*5/HAR3	4.65 ± 0.45a	24 ± 7.67a	0.669 ± 0.1a	122 ± 1.21a
HA89*5/HAR2	4.40 ± 0.45a	21 ± 7.67a	0.552 ± 0.1a	123 ± 1.21a
Allopurinol				
Control (0)	7.41 ± 0.39a	35 ± 7.62a	1.257 ± 0.1a	123 ± 1.18a
50µM	3.34 ± 0.39b	21 ± 7.62b	0.492 ± 0.1b	122 ± 1.18a
100µM	1.11 ± 0.39c	12 ± 7.62c	0.181 ± 0.1a	123 ± 1.18a

Where the number to the right of ± represents the mean while to the left is the standard error.

Mean values with different letters are significantly different ($P < 0.05$, least significant difference).

Table 3.2b: Plant height, weight of stalks, area of head and weight of head associated with race, genotype and allopurinol main effects

Factor	Plant height (cm)	Weight of stalks (g)	Area of head (cm^2)	Weight of head (g)
Race				
Control (0)	125.9 ± 12a	33.78 ± 0.6b	55.96 ± 1.3a	25.49 ±0.6a
304	123.4 ± 12a	31.96 ± 0.6c	50.18 ± 1.3b	24.37 ± 0.6a
336	124.5 ± 12a	37.21 ± 0.6a	57.81 ± 1.3a	25.43 ± 0.6a
Genotype				
HA89	123.2 ± 13bc	34.40 ± 0.6a	55.59 ± 1.5a	25.64 ± 0.8a
HA89*5/cm90	126.3 ± 13ab	34.71 ± 0.6a	52.76 ± 1.5a	24.59 ± 0.8a
HA89*5/HAR3	129.3 ± 13a	34.39 ± 0.6a	54.93 ± 1.5a	25.10 ± 0.8a
HA89*5/HAR2	119.6 ± 13c	33.72 ± 0.6a	55.31 ± 1.5a	25.07 ± 0.8a
Allopurinol				
Control (0)	125.6 ± 13a	34.82 ± 0.6a	57.3 ± 1.3a	25.75 ± 0.7a
50µM	124.4 ± 13a	34.55 ± 0.6a	54.3 ± 1.3ab	24.32 ± 0.7a
100µM	123.8 ± 13a	33.59 ± 0.6a	52.5 ± 1.3b	25.24 ± 0.7a

Where the number to the right of ± represents the mean while to the left is the standard error.

Mean values with different letters are significantly different ($P < 0.05$, least significant difference).

Table 3.2c: Number of seeds, weight of seeds, weight of biomass and harvest index associated with race, genotype and allopurinol main effects

Factor	Number of seeds	Weight of seeds (g)	Weight of biomass (g)	Harvest index
Race				
Control (0)	309 ± 8.8b	12.41 ± 0.3a	59.34 ± 0.9b	0.19 ± 0.01a
304	264 ± 8.8c	10.72 ± 0.3b	56.05 ± 0.9c	0.17 ± 0.01b
336	331 ± 8.8a	12.35 ± 0.3a	62.88 ± 0.9a	0.18± 0.01ab
Genotype				
HA89	187 ± 10.2bc	11.9 ± 0.4ab	59.8 ± 1.1a	0.18 ± 0.01a
HA89*5/cm90	312 ± 10.2ab	11.8 ± 0.4ab	59.4 ± 1.1a	0.18 ± 0.01a
HA89*5/HAR3	330 ± 10.2a	12.5 ± 0.4a	60.2 ± 1.1a	0.18 ± 0.01a
HA89*5/HAR2	227 ± 10.2c	11.1 ± 0.4b	58.3 ± 1.1a	0.17 ± 0.01a
Allopurinol				
Control (0)	317 ± 8.8a	12.7 ± 0.3a	60.8 ± 0.9a	0.18 ± 0.01a
50µM	305 ± 8.8a	11.7 ± 0.3b	59.03± 0.9ab	0.18 ± 0.01a
100µM	282 ± 8.8b	11.2 ± 0.3b	58.39 ± 0.9b	0.17 ± 0.01a

Where the number to the right of ± represents the mean while to the left is the standard error.

Mean values with different letters are significantly different ($P < 0.05$, least significant difference).

Table 3:3 Correlations between traits in sunflower

	PDSA	NOLE	NDRS	DMY	PTHT	SKTW	ARHD	HDWT	SDWT	SDNO	BMS	HI
PDSA	1.0000											
NOLE	0.5438 *<.0001*	1.0000										
NDRS	0.5913 *<.0001*	0.5800 *<.0001*	1.0000									
DMY	0.0765 *0.1121ns*	-0.0784 *0.1037ns*	-0.3720 *0.4405ns*	1.0000								
PTHT	0.0170 *0.7246ns*	-0.1482 *0.0020*	0.0523 *0.2784ns*	0.1713 *0.0003*	1.0000							
SKWT	0.0655 *0.1740ns*	-0.1823 *0.0001*	0.0397 *0.4101ns*	0.1469 *0.0022*	0.7325 *<.0001*	1.0000						
ARHD	0.0845 *0.0792ns*	-0.1825 *0.0001*	0.0595 *0.2173ns*	0.1667 *0.0005*	0.6736 *<.0001*	0.8559 *<.0001*	1.0000					
HDWT	0.0495 *0.3047ns*	-0.1874 *<.0001*	0.0172 *0.7215ns*	0.1948 *<.0001*	0.6318 *<.0001*	0.8149 *<.0001*	0.8774 *<.0001*	1.0000				
SDWT	0.0398 *0.4093ns*	-0.1744 *0.0003*	0.0229 *0.6340ns*	0.1289 *0.0073*	0.6676 *<.0001*	0.8485 *<.0001*	0.8987 *<.0001*	0.9013 *<.0001*	1.0000			
SDNO	0.0584 *0.2258ns*	-0.1542 *0.0013*	0.0078 *0.8714ns*	0.0699 *0.1466ns*	0.6803 *<.0001*	0.8467 *<.0001*	0.8523 *<.0001*	0.8224 *<.0001*	0.9204 *<.0001*	1.0000		
BMS	0.0619 *0.1991ns*	-0.1931 *<.0001*	0.0321 *0.5052ns*	0.1741 *0.0003*	0.7251 *<.0001*	0.9698 *<.0001*	0.9057 *<.0001*	0.9317 *<.0001*	0.9112 *<.0001*	0/8768 *<.0001*	1.0000	
HI	0.0325 *0.5000ns*	-0.0100 *0.3700ns*	0.0247 *0.6167ns*	0.0223 *0.644ns*	0.4599 *<.0001*	0.5093 *<.0001*	0.6902 *<.0001*	0.6465 *<.0001*	0.8316 *<.0001*	0.7387 *<.0001*	0.5913 *<.0001*	1.0000

Key: PDSA = Percent disease area, NOLE = number of lesions, NDRS = NDSU disease severity rating score, DMY = days to maturity, PTHT = plant height, SKWT = weight of stalks, ARHD = area of head, HDWT = weight of head, SDWT = weight of seeds, SDNO = number of seeds, BMS = weight of biomass and HI = harvest index. Italicized numbers are the P-values indicating significance or not significance (ns).

Fig. Legends

Fig. 3.1. Race × genotype interaction effects on percent disease area. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 3.2. Race × genotype interaction effects on days to maturity. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 3.3. Race × allopurinol interaction effects on number of seeds. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 3.4. Race × allopurinol interaction effects on percent disease area. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 3.5. Race × allopurinol interaction effects on number of lesions. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 3.6. Race × allopurinol interaction effects on NDSU rating score. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 3.7. Race × allopurinol interaction effects on days to maturity. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.8. Race × allopurinol interaction effects on weight of stalks. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.9. Race × allopurinol interaction effects on area of head. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.10. Race × allopurinol interaction effects on weight of head. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.11. Race × allopurinol interaction effects on number of seeds. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.12. Race × allopurinol interaction effects on weight of seeds. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.13. Race × allopurinol interaction effects on weight of biomass. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.14. Race × allopurinol interaction effects on harvest index. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.15. Genotype × allopurinol interaction effects on number of seeds. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.16. Genotype × allopurinol interaction effects on weight of seeds. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.17. Genotype × allopurinol interaction effects on harvest index. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.18. Race × genotype × allopurinol interaction effects on percent disease area. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.19. Race × genotype × allopurinol interaction effects on weight of stalks. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.20. Race × genotype × allopurinol interaction effects on weight of heads. Error bars are standard errors of means. Error bars with different letters are significantly different (P<0.05, least significant difference).

Fig. 3.21. Race × genotype × allopurinol interaction effects on number of seeds. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 3.22. Race × genotype × allopurinol interaction effects on weight of seeds. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 3.23. Race × genotype × allopurinol interaction effects on weight of biomass. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 3.24. Race × genotype × allopurinol interaction effects on harvest index. Error bars are standard errors of means. Error bars with different letters are significantly different ($P<0.05$, least significant difference).

Fig. 3.25a. Disease reaction of genotypes to pathogen inoculations under different doses of allopurinol

Fig. 3.25b. Disease reaction of genotypes to pathogen inoculations under different dose of allopurinol

Fig. 3.1.

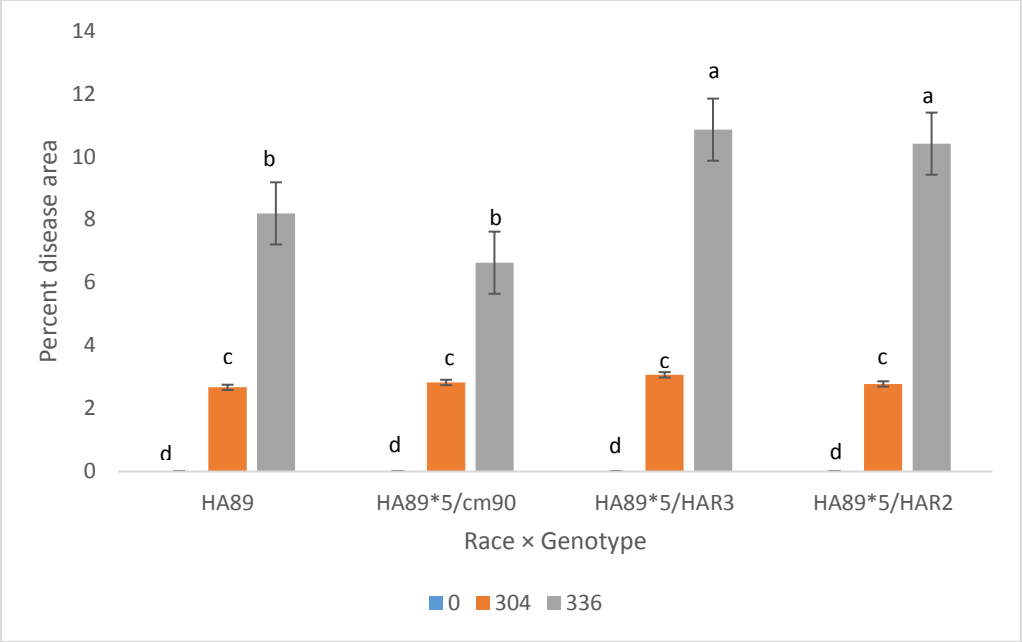

14
12
10
8
6
4
2
0
Percent disease area
d
c
b
d
c
b
d
c
a
d
c
a
HA89
HA89*5/cm90
HA89*5/HAR3
HA89*5/HAR2
Race × Genotype
0
304
336

Fig. 3.2.

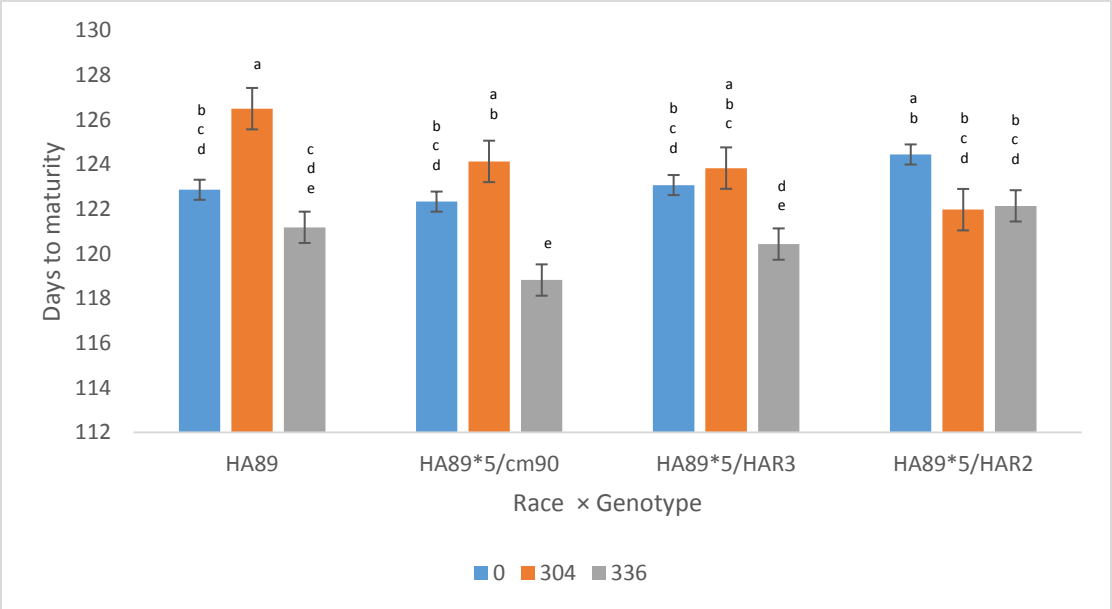

130
128
126
124
122
120
118
116
114
112
Days to maturity
bcd
a
cde
bcd
ab
e
bcd
abc
de
ab
bcd
bcd
HA89
HA89*5/cm90
HA89*5/HAR3
HA89*5/HAR2
Race × Genotype
0
304
336

Fig. 3.3.

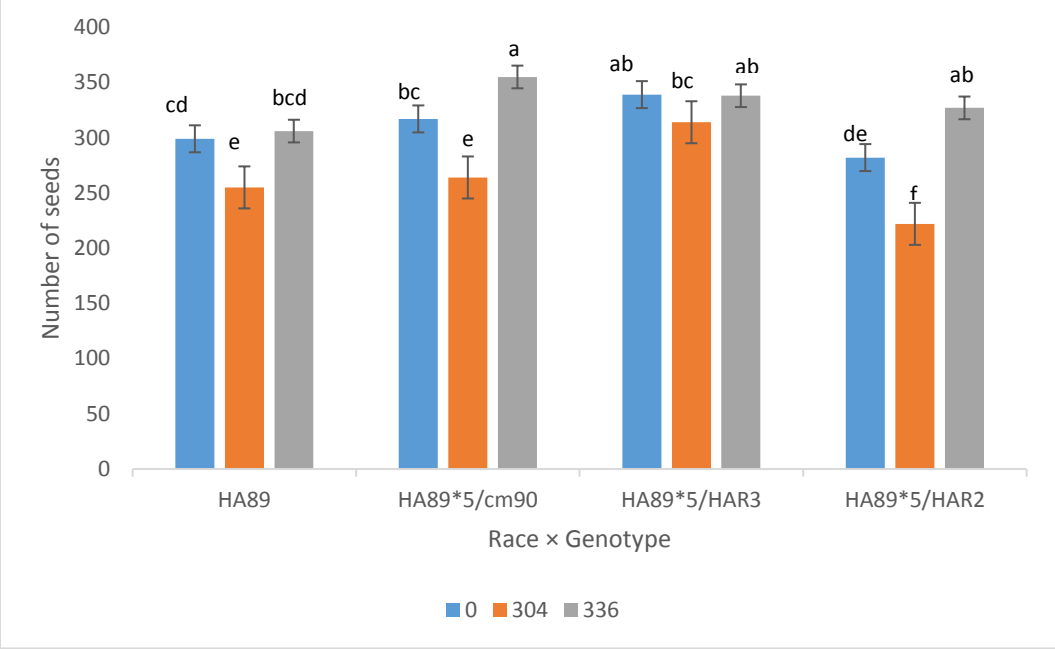

400
350
300
250
200
150
100
50
0
Number of seeds
cd
e
bcd
bc
e
a
ab
bc
ab
de
f
ab
HA89
HA89*5/cm90
HA89*5/HAR3
HA89*5/HAR2
Race × Genotype
0
304
336

Fig. 3.4.

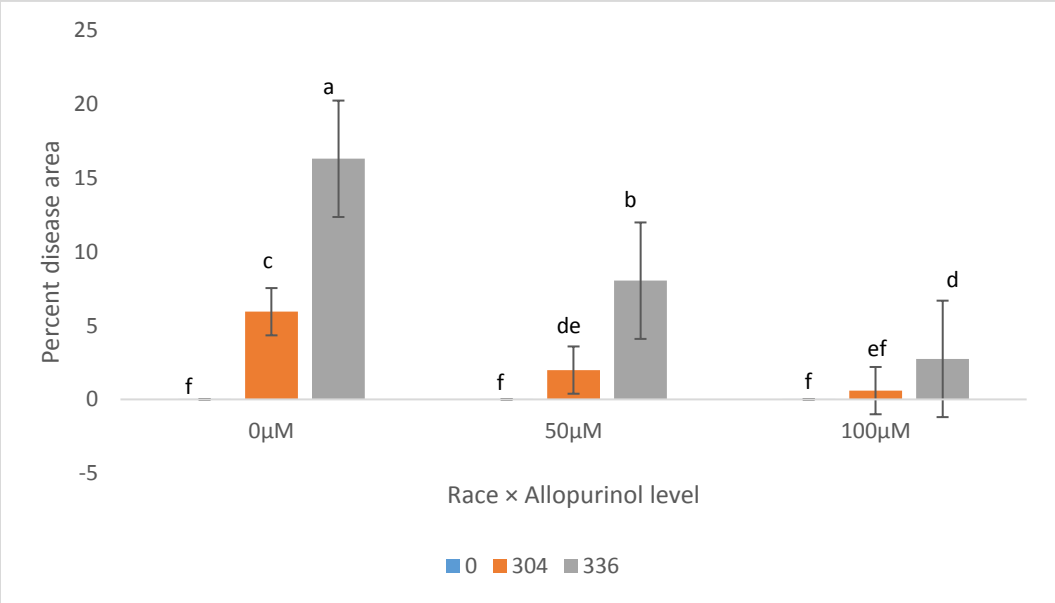

25
20
15
10
5
0
-5
Percent disease area
f
c
a
f
de
b
f
ef
d
0µM
50µM
100µM
Race × Allopurinol level
0
304
336

Fig. 3.5.

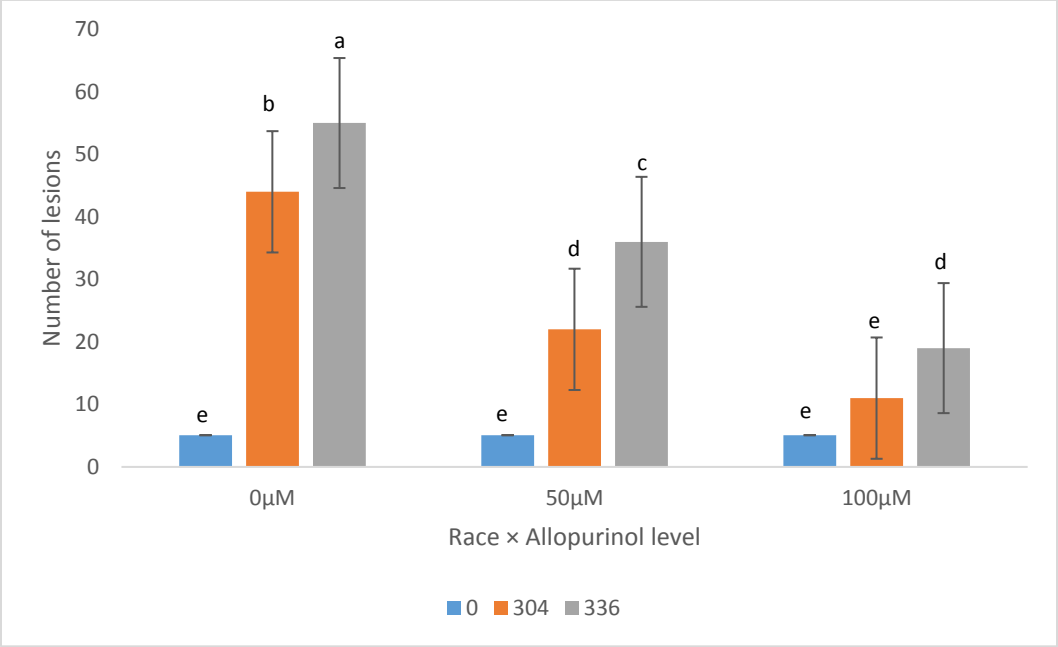

70
60
50
40
30
20
10
0
Number of lesions
a
b
c
d
e
d
e
e
e
0µM
50µM
100µM
Race × Allopurinol level
0
304
336

Fig. 3.6.

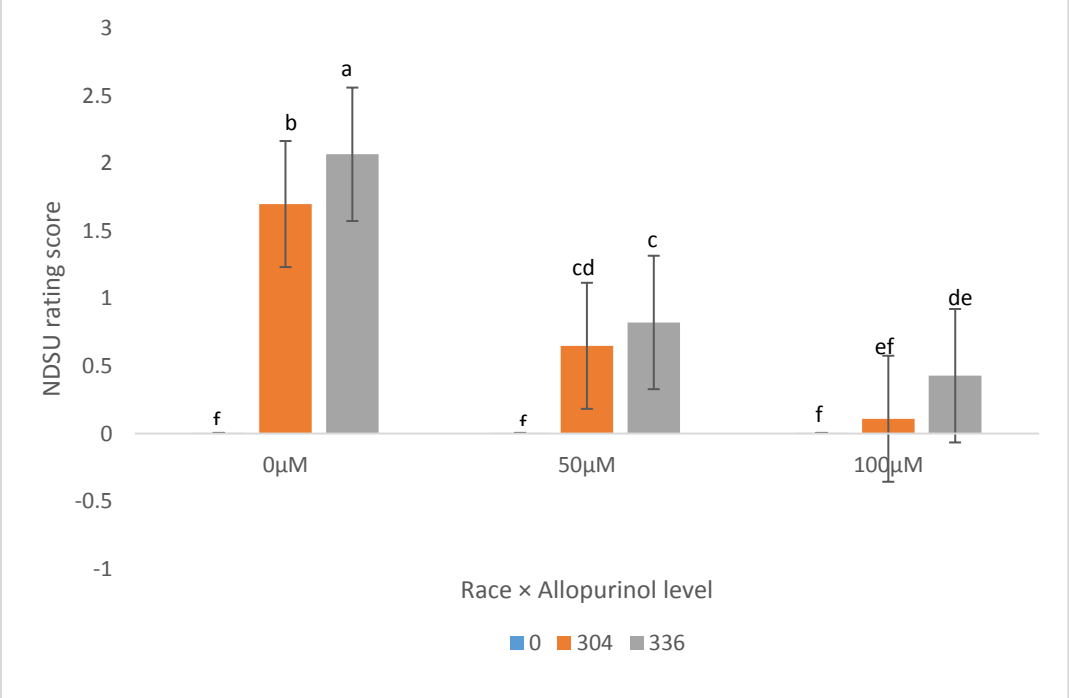

3
2.5
2
1.5
1
0.5
0
-0.5
-1
NDSU rating score
a
b
c
cd
de
ef
f
f
f
0µM
50µM
100µM
Race × Allopurinol level
0
304
336

Fig. 3.7

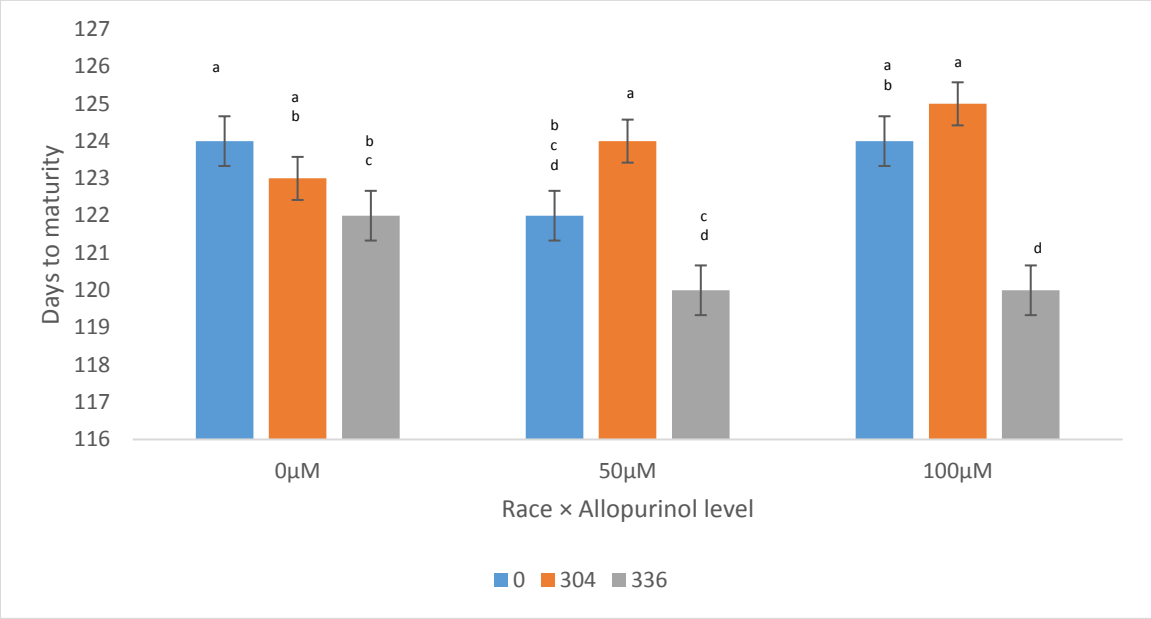

Days to maturity
127
126
125
124
123
122
121
120
119
118
117
116
a
a b
b c
b c d
a
c d
a b
a
d
0µM
50µM
100µM
Race × Allopurinol level
0
304
336

Fig. 3.8

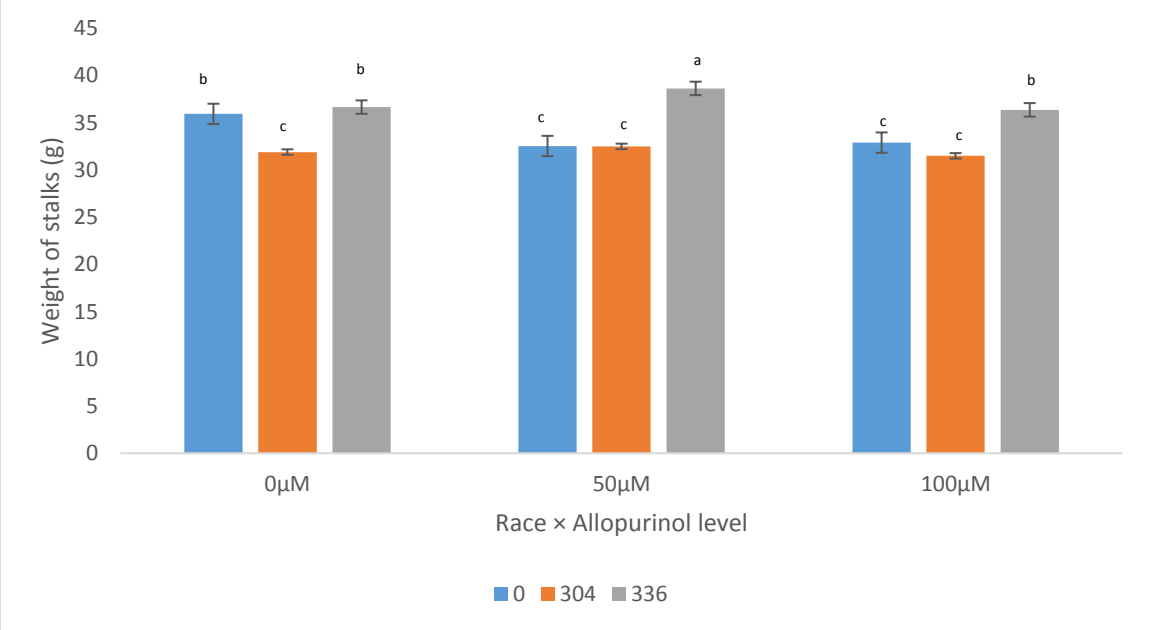

Weight of stalks (g)
45
40
35
30
25
20
15
10
5
0
b
c
b
c
c
a
c
c
b
0µM
50µM
100µM
Race × Allopurinol level
0
304
336

Fig. 3.9

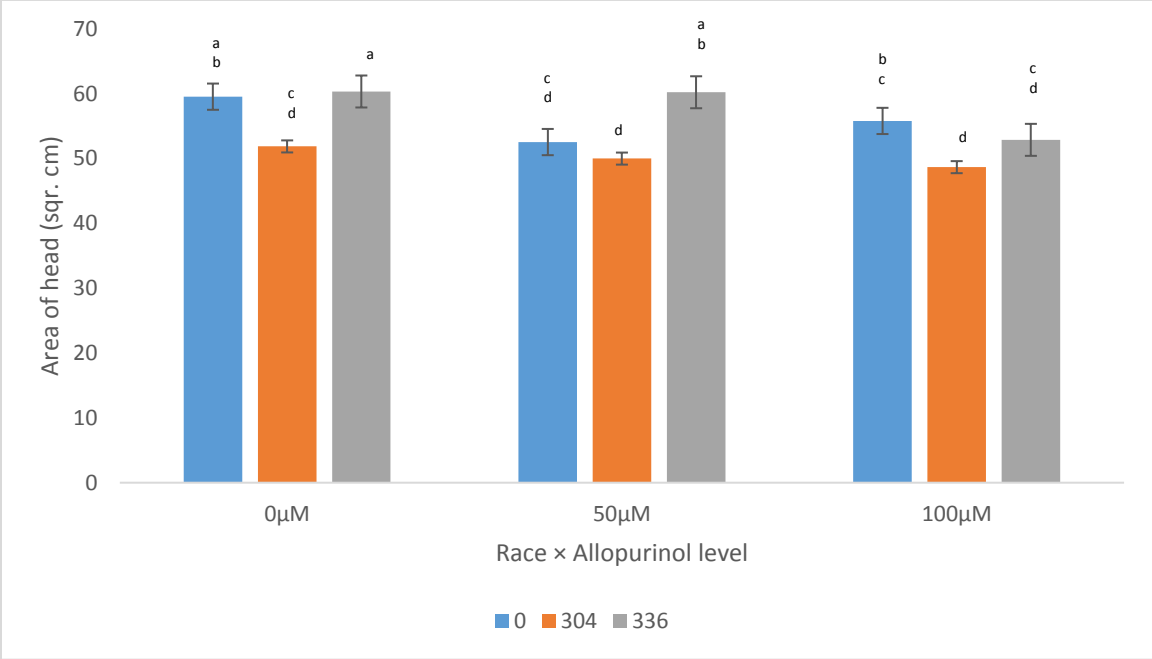


Fig. 3.10

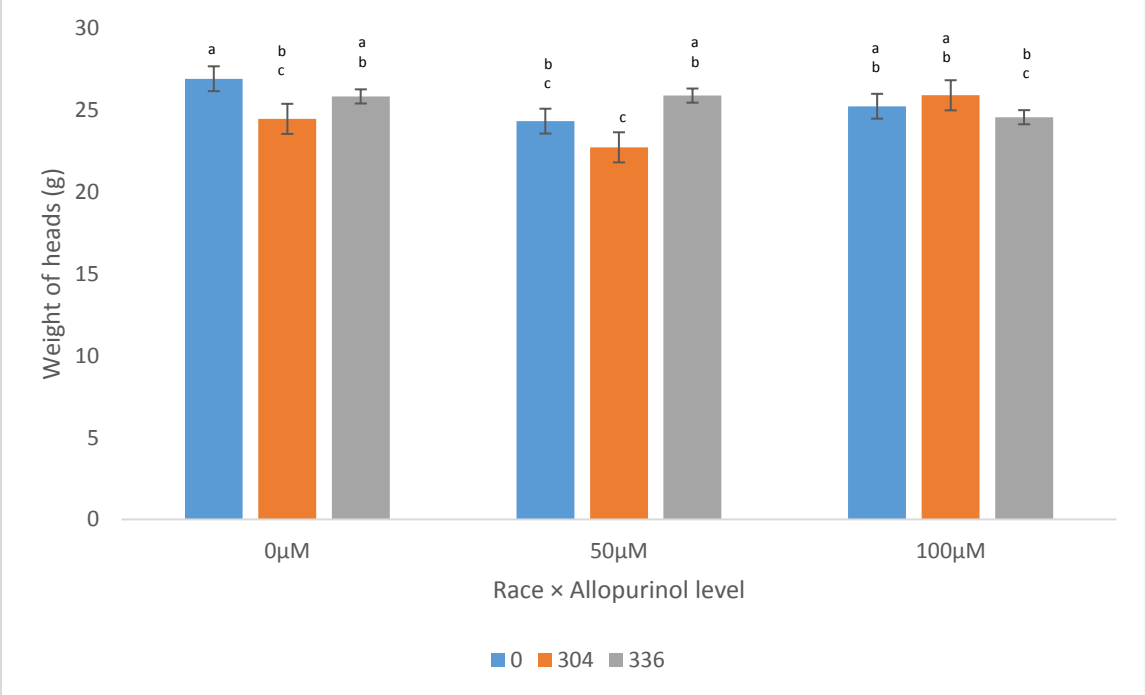


Fig. 3.11

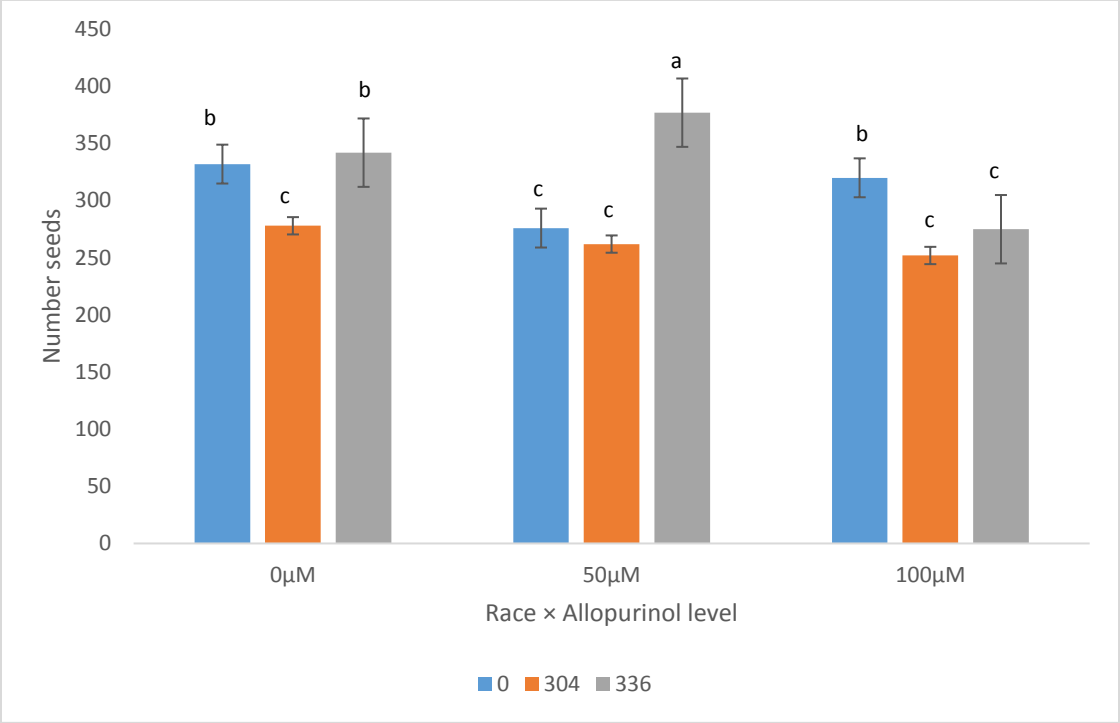


Fig. 3.12.

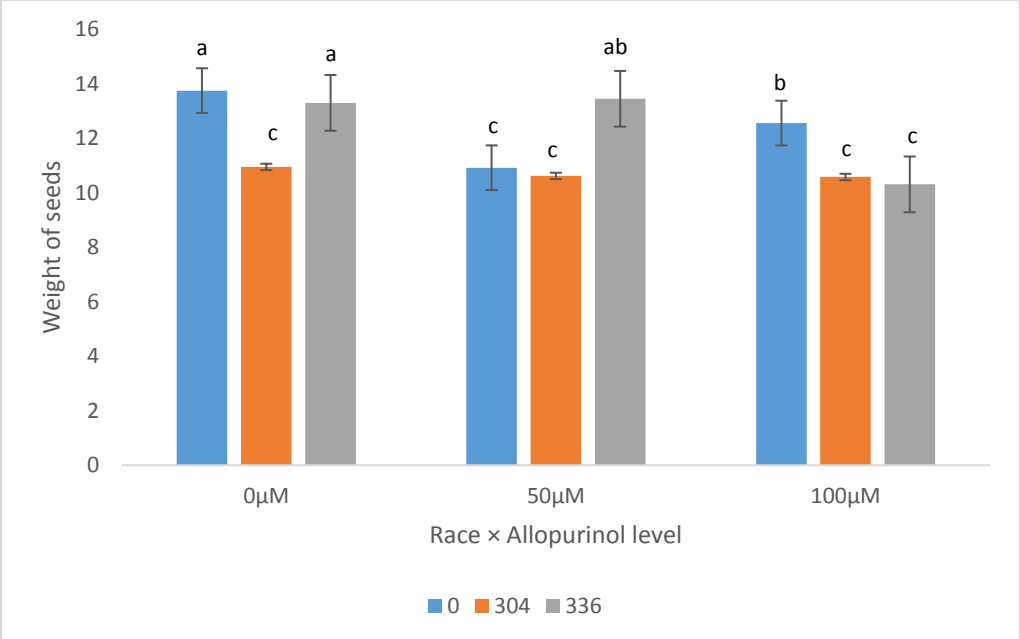


Fig. 3.13

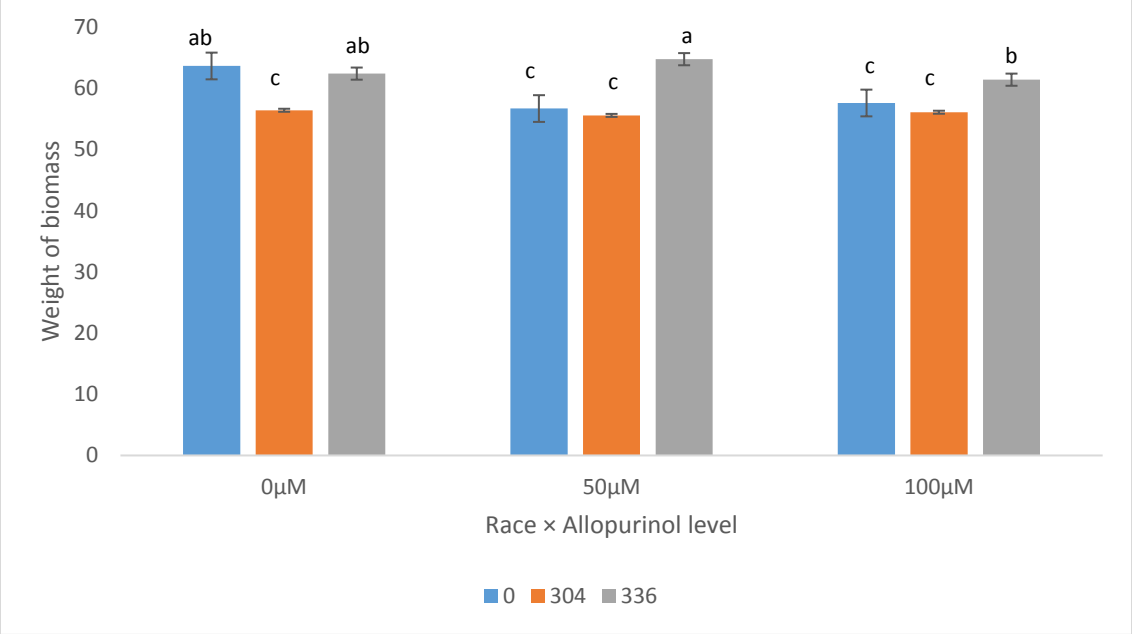


Fig. 3.14

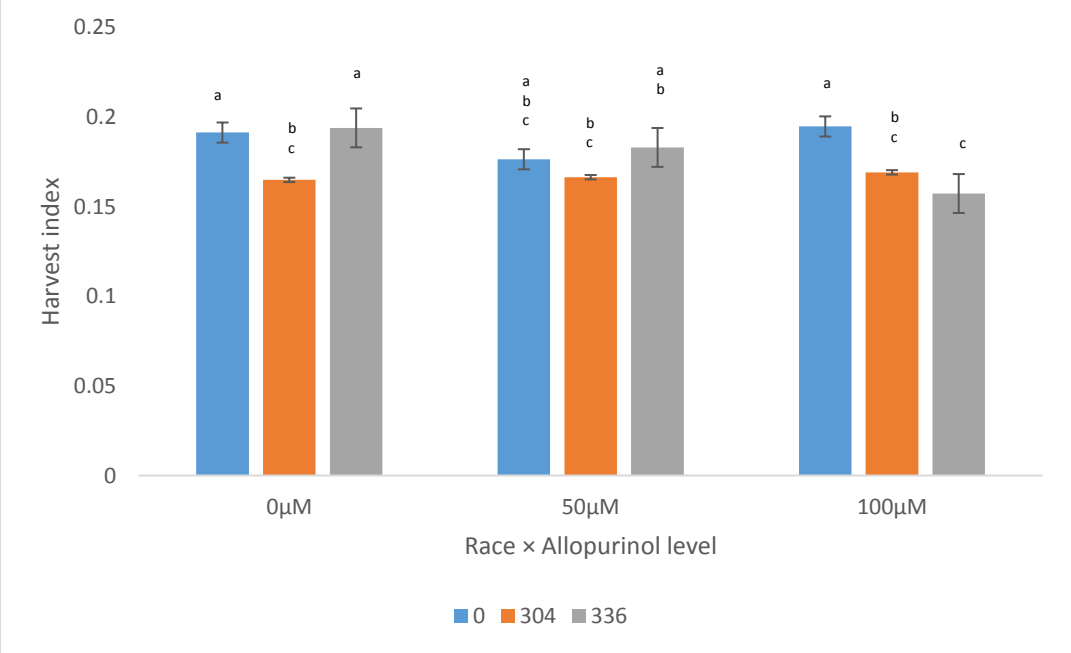


Fig. 3.15

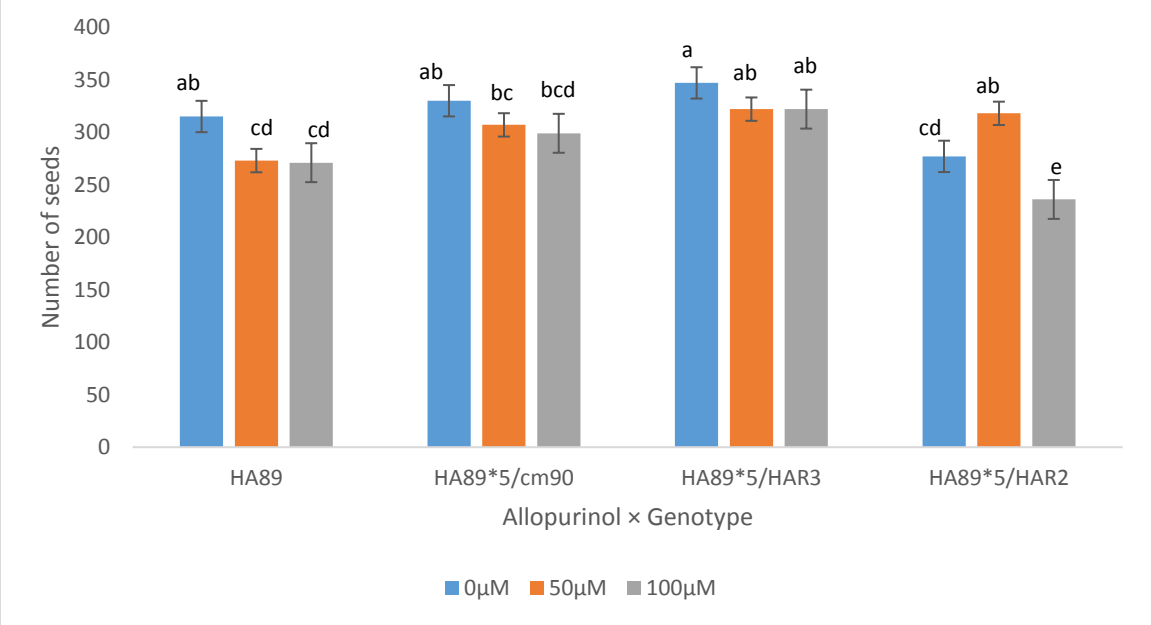


Fig. 3.16

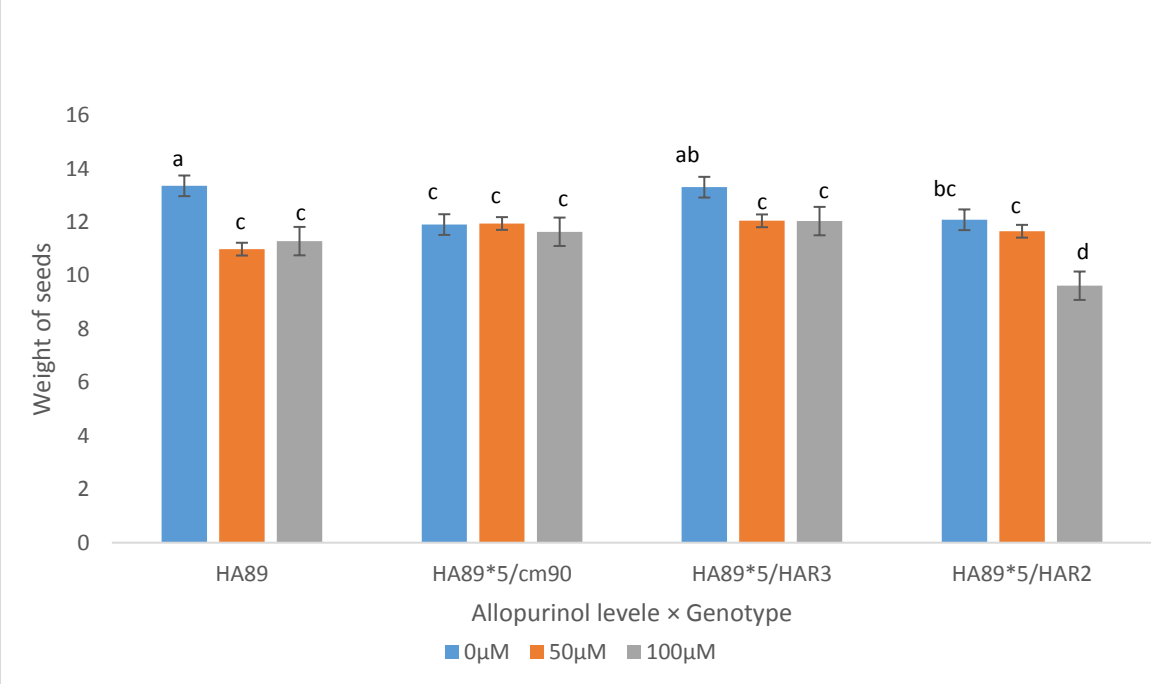


Fig. 3.17

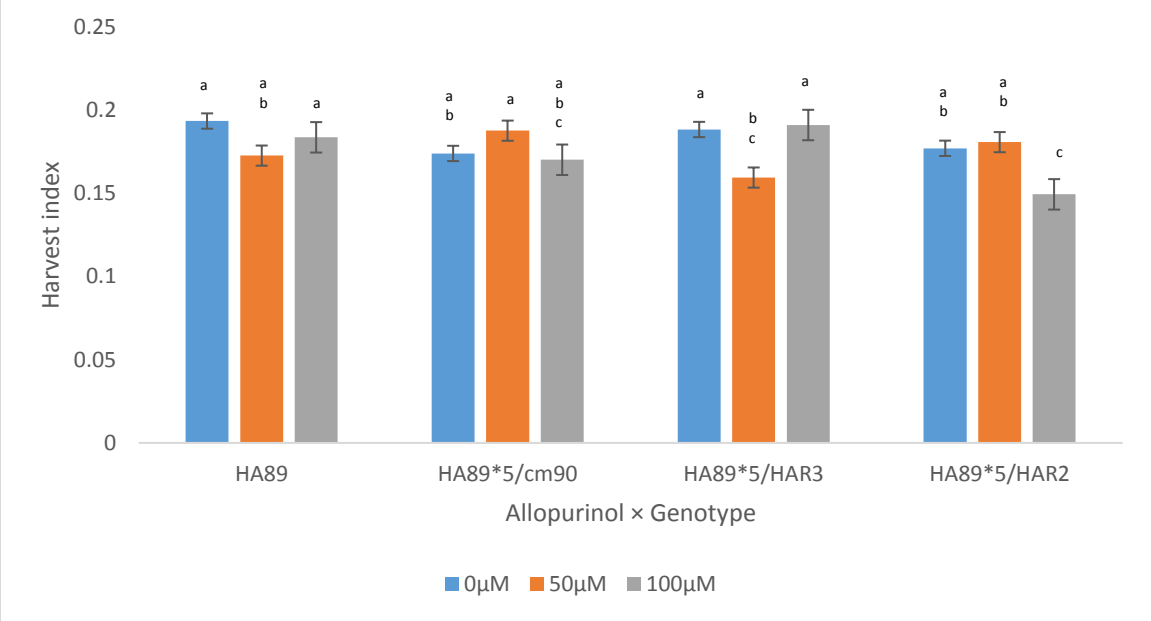


Fig. 3.18

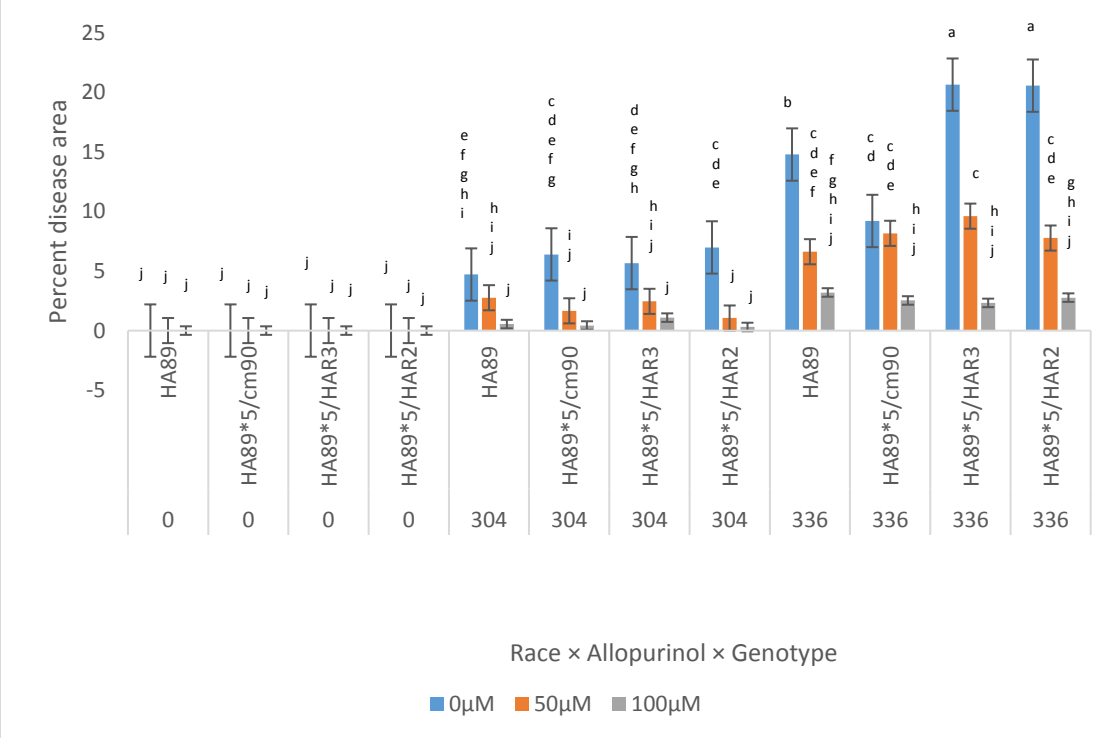


Fig. 3.19.

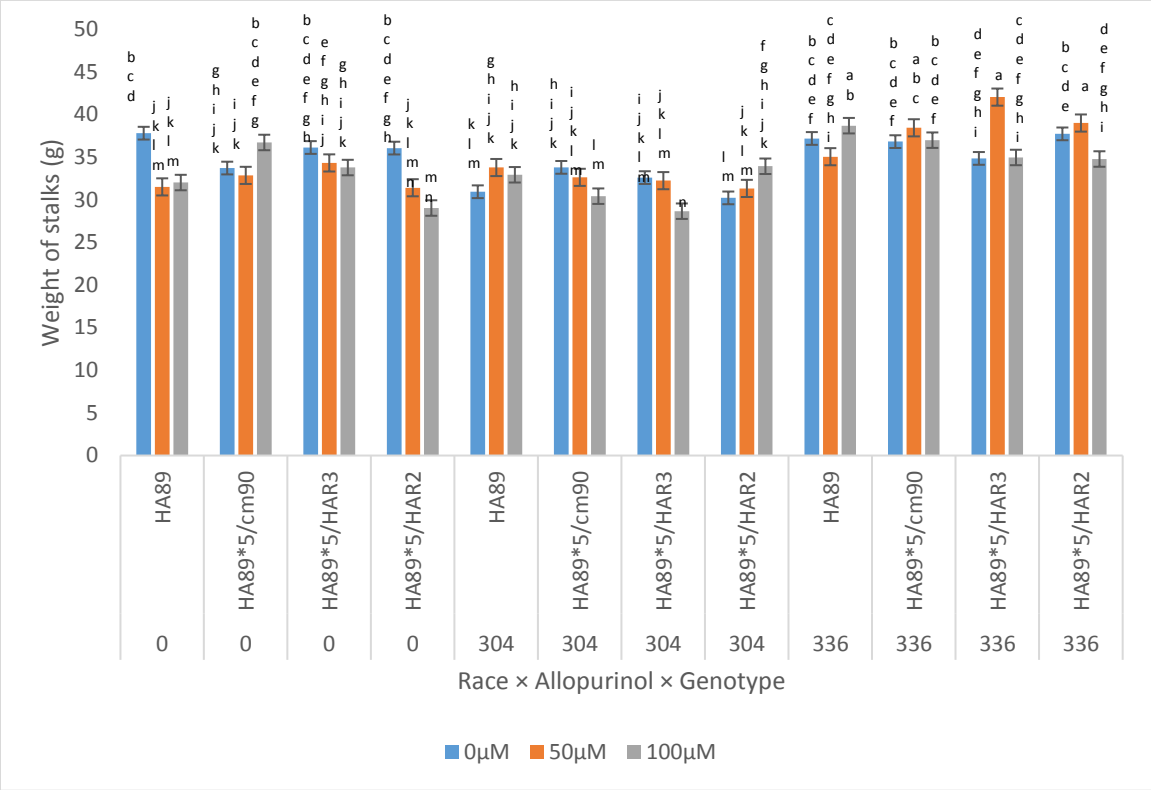

Weight of stalks (g)
50
45
40
35
30
25
20
15
10
5
0
HA89
HA89*5/cm90
HA89*5/HAR3
HA89*5/HAR2
HA89
HA89*5/cm90
HA89*5/HAR3
HA89*5/HAR2
HA89
HA89*5/cm90
HA89*5/HAR3
HA89*5/HAR2
0
0
0
0
304
304
304
304
336
336
336
336
Race × Allopurinol × Genotype
0µM
50µM
100µM

Fig. 3.20

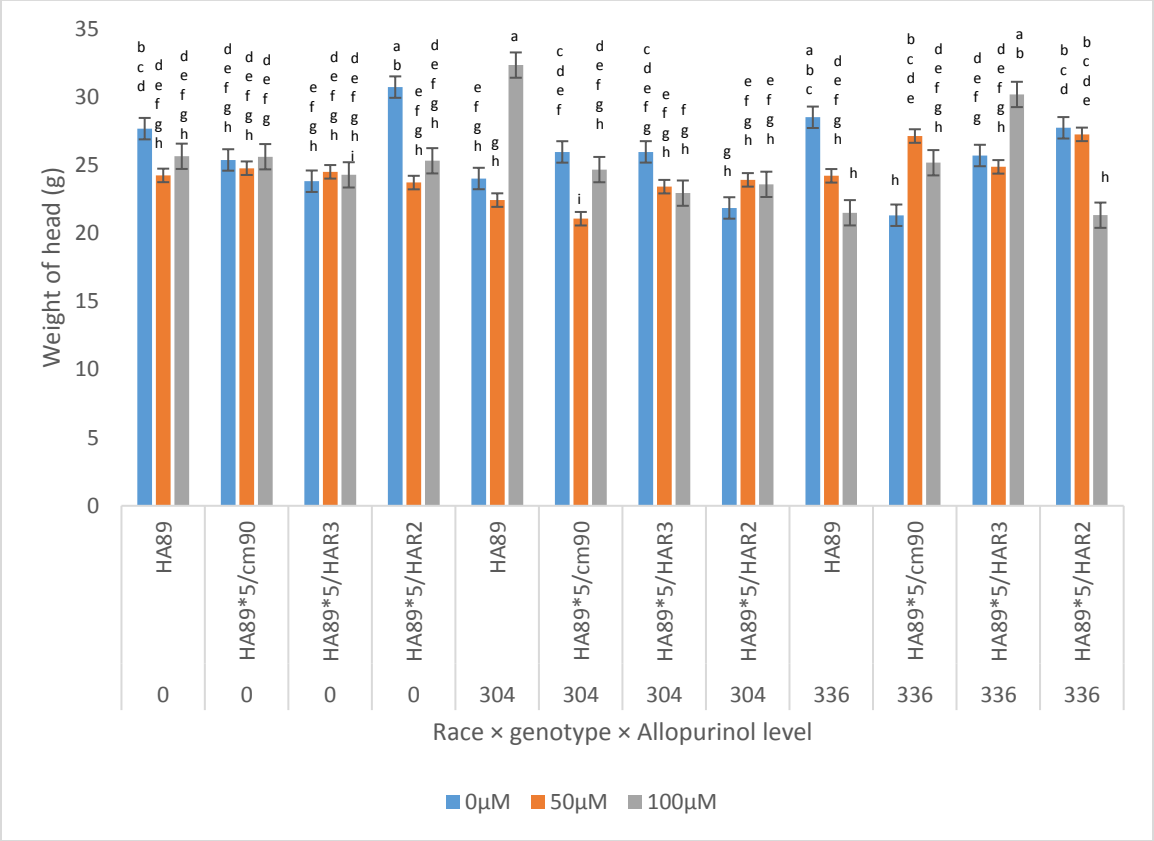

Weight of head (g)
35
30
25
20
15
10
5
0
HA89
HA89*5/cm90
HA89*5/HAR3
HA89*5/HAR2
0
304
336
Race × genotype × Allopurinol level
0µM
50µM
100µM

Fig. 3.21

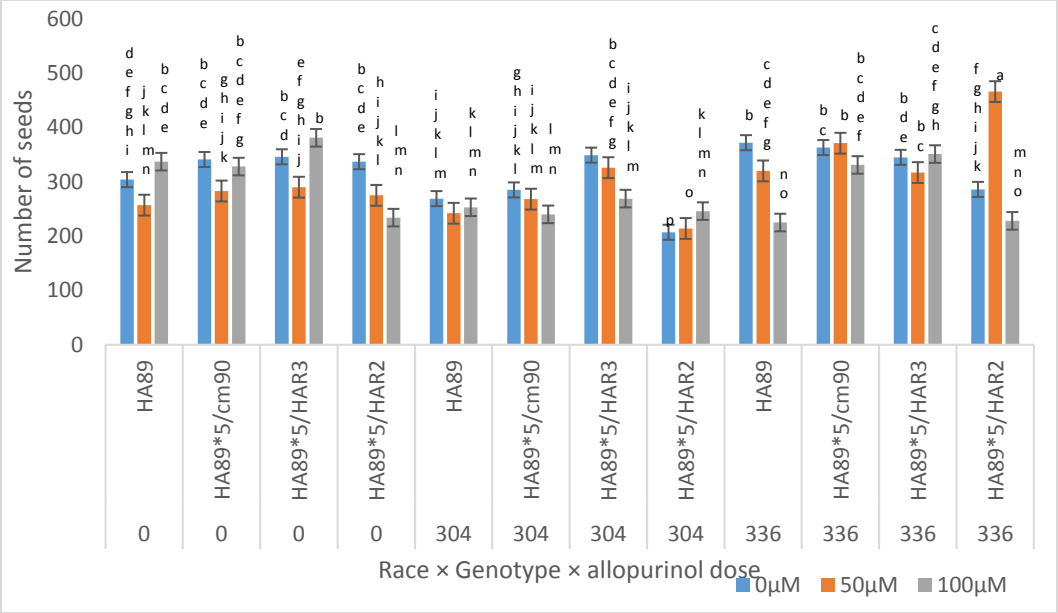
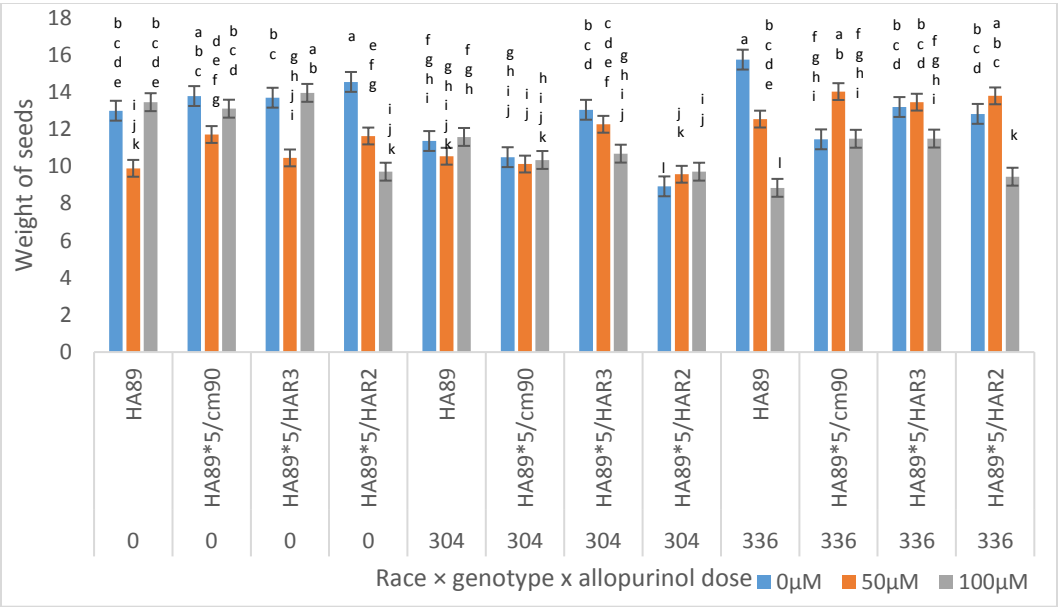


Fig. 3.22



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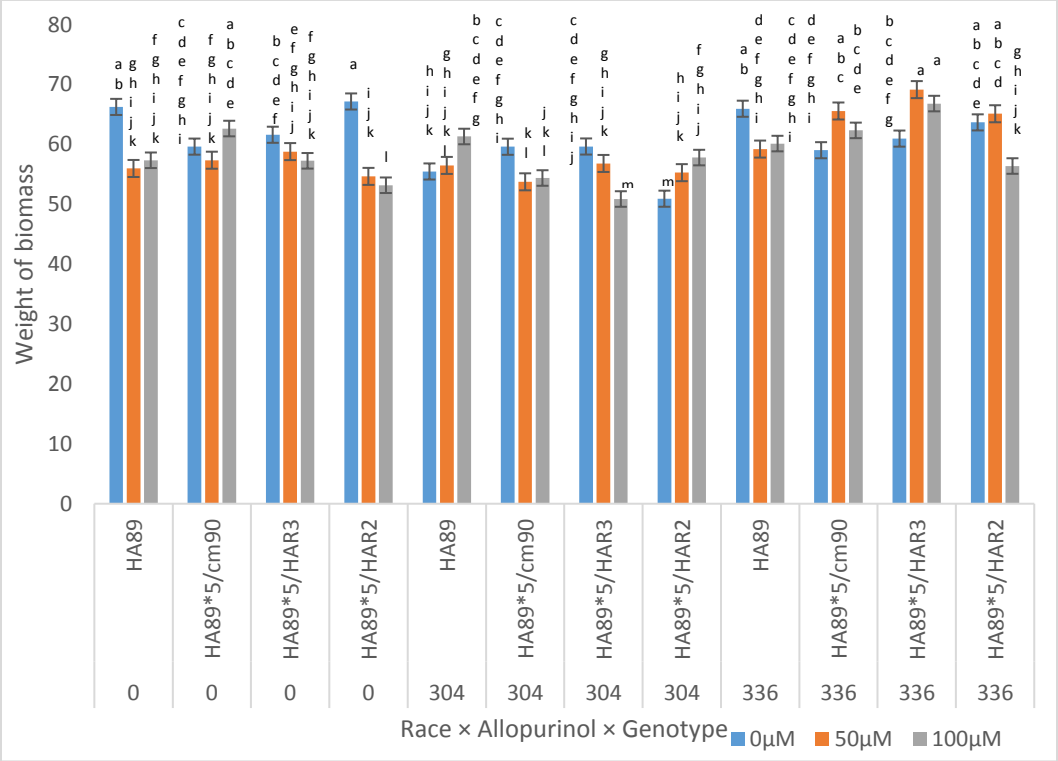

Weight of biomass
80
70
60
50
40
30
20
10
0
HA89
HA89*5/cm90
HA89*5/HAR3
HA89*5/HAR2
0
304
336
Race × Allopurinol × Genotype
0µM
50µM
100µM

Fig. 3.24

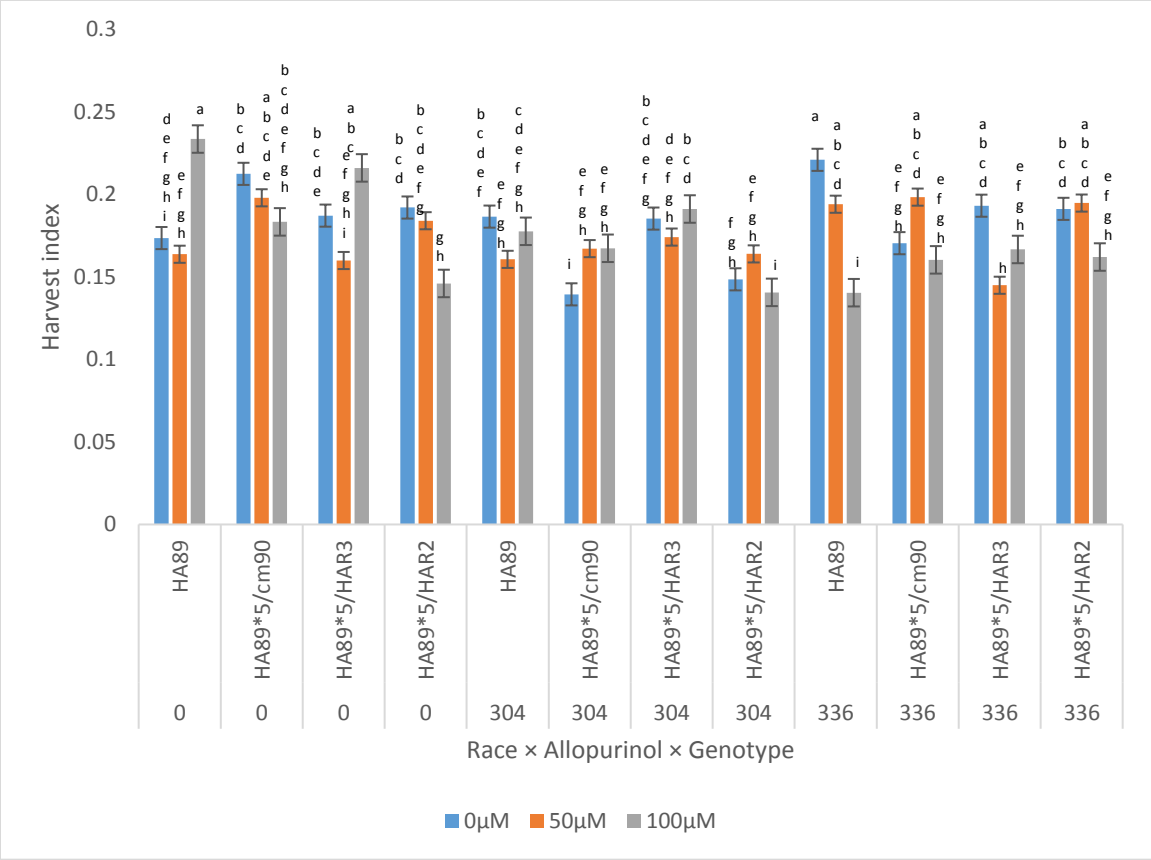
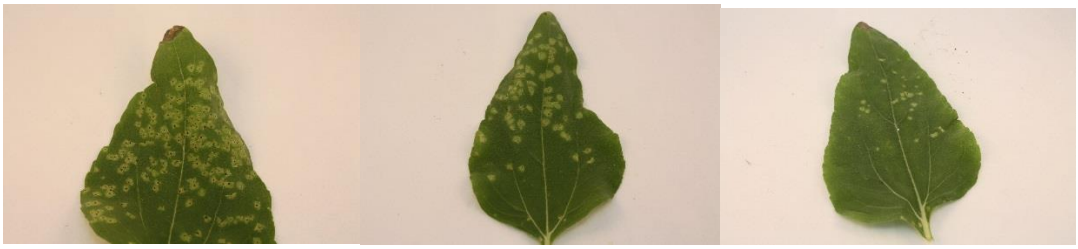

Harvest index
0.3
0.25
0.2
0.15
0.1
0.05
0
HA89
HA89*5/cm90
HA89*5/HAR3
HA89*5/HAR2
HA89
HA89*5/cm90
HA89*5/HAR3
HA89*5/HAR2
HA89
HA89*5/cm90
HA89*5/HAR3
HA89*5/HAR2
0
0
0
0
304
304
304
304
336
336
336
336
Race × Allopurinol × Genotype
0μM
50μM
100μM

Fig. 3.25a

304 race: HA89 × 0μM **HA89 × 50μM** **HA89 × 100μM**

336 race: HA89 × 0μM **HA89 × 50μM** **HA89 × 100μM**



304 race: HA89*5/cm90 × 0μM **HA89*5/cm90 × 50μM** **HA89*5/cm90 × 100μM**

336 race: HA89*5/cm90 × 0μM **HA89*5/cm90 × 50μM** **HA89*5/cm90 × 100μM**

Fig. 3.25b

304 race: HA89*5/HAR3 × 0μM **HA89*5/HAR3 × 50μM** **HA89*5/HAR3 × 100μM**

336 race: HA89*5/HAR3 × 0μM **HA89*5/HAR3 × 50μM** **HA89*5/HAR3 × 100μM**

304 race: HA89*5/HAR2 × 0μM **HA89*5/HAR2 × 50μM** **HA89*5/HAR2 × 100μM**

336 race: HA89*5/HAR2 × 0μM **HA89*5/HAR2 × 50μM** **HA89*5/HAR2 × 100μM**

CHAPTER 4: GENERAL CONCLUSIONS AND RECOMMENDATIONS

The results from the studies have unraveled some information on the effects of allopurinol chemical on lesion mimic mutant of wheat and in near isogenic lines of sunflower. Under wheat experimentation, the study focused on the effects of allopurinol chemical on a lesion mimic mutant that constitutively produces hypersensitive flecks from heading growth stage. The flecks are a physiological response because of mutations within its genome, which produced a recessive *lm* gene. The *lm* gene induces hypersensitive reactions constitutively that the plant may take advantage in conferring diseases in situations where the pathogens prevail. However, in situations where the pathogen is not present then the use of the mechanism is costly to the plant since hypersensitive reaction process is energy demanding. The plant may utilize the resources for defense in the absence of a pathogen instead deploying the photosynthates to the heads for grain filling and subsequently increasing the number and seed weight. The results from the study showed that higher levels of allopurinol chemical were effective in reducing the chlorotic area, percent chlorotic area relative to the total leaf sampled area and number of lesions. This shows the effectiveness of allopurinol chemical in inhibiting the activities of xanthine oxidase that promote production of reactive oxygen species that induce cell death in tissues. On the other hand, application of allopurinol chemical came with a penalty cost to the host. High dosages negatively reduced the chlorophyll content in the leaves, reduced number of heads, weight of heads, grain weight and number of seed traits. The results suggest that interference with xanthine oxidase and subsequently reactive oxygen species may have huge consequences on yield and yield components;

therefore, there precautionary decisions be made before interfering with xanthine oxidase as a technique in minimizing constitutive hypersensitive responses in host raised in disease free environments. The study did not use any wet chemistry studies, therefore there is a need for studies in future to incorporate wet chemistry to tie up the loose ends identified in the study.

Sunflower rust is one of the major diseases causing huge biological and economic yield losses. The discovery and deployment of R-genes is an ongoing breeding strategy in the sunflower industry. However, the existence of coevolutionary relationship between the pathogens and hosts create an environment where the pathogen develops virulent mechanisms in overcoming any resistance in a host. In the study, near isogenic lines of sunflower were inoculated with sunflower races that are aggressive and prevalent in sunflower growing areas. Furthermore, some treatments received different rates of allopurinol chemical as a soil drench. The results from the study showed that high rates of allopurinol effectively reduced disease symptoms in all the inoculated plants. Thus, there was a reduction in percent disease area, number of lesions and disease severity rating scores was low under high allopurinol dose. The results suggest that the allopurinol treatment created unfavorable conditions in the host hence disrupted the pathogen from multiplying and causing more disease symptoms than in the control. Despite allopurinol chemical effectively reduced the pathogenicity of races, there was a penalty cost inflicted on the host on yield and yield component traits. Higher levels of allopurinol treatments reduced the area of heads, number of seeds, weight of seeds and weight of biomass. All these traits play a role in determining the yield potential of a host. Therefore, use allopurinol chemical as a potential compound in disease control strategy

has to be taken on board with some precautionary measures looking at the penalty cost inflicted on the yield traits.

In future, there will be a need to reconsider inoculating the plants at different stages during growth and development of the plants since it was very difficult to relate the responses captured at early stages to yield and yield components traits associated with disease data. Thus, inoculation of the hosts in later stages of growth and early reproductive phases may fill the gaps observed in the study. Furthermore, there will be a need to conduct some wet chemistry to fill up the gaps on the biochemical activities that took place in the host under pathogen, host and allopurinol environment.

LIST OF APPENDICES

Appendix 3.1. Percent disease area, number of lesions and NDSU rating score associated with race × genotype interactions

Race × genotype interaction	Percent disease area	Number of lesions	NDSU rating score
336 × HA89*5/HAR3	10.86 ± 0.76a	39 ± 8.06a	1.114 ± 0.17ab
336 × HA89*5/HAR2	10.41 ± 0.76a	37 ± 8.06ab	1.007 ± 0.17abc
336 × HA89	8.19 ± 0.76b	36 ± 8.06ab	0.979 ± 0.17abc
336 × HA89*5/cm90	6.63 ± 0.76b	37 ± 8.06ab	1.369 ± 0.17a
304 × HA89*5/HAR3	3.07 ± 0.76c	26 ± 8.06c	0.887 ± 0.17bc
304 × HA89*5/cm90	2.83 ± 0.76c	29 ± 8.06bc	0.769 ± 0.17bc
304 × HA89*5/HAR2	2.78 ± 0.76c	22 ± 8.06c	0.643 ± 0.17c
304 × HA89	2.67 ± 0.76c	26 ± 8.06c	0.005 ± 0.17d
0 × HA89*5/HAR2	0.01 ± 0.76d	5 ± 8.06d	0.005 ± 0.17d
0 × HA89	0.01 ± 0.76d	5 ± 8.06d	0.005 ± 0.17d
0 × HA89*5/HAR3	0.01 ± 0.76d	5 ± 8.06d	0.005 ± 0.17d
0 × HA89*5/cm90	0.01 ± 0.76d	5 ± 8.06d	0.005 ± 0.17d

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.2. Percent disease area, number of lesions and NDSU rating score associated with race × allopurinol interactions

Race × allopurinol dose interaction	Percent disease area	Number of lesions	NDSU rating score
336 × 0µM	16.29 ± 0.66 a	56 ± 7.91a	2.067 ± 0.16a
336 × 50µM	8.04 ± 0.66b	36 ± 7.91c	0.822 ± 0.16c
336 × 100µM	2.74 ± 0.66d	19 ± 7.91d	0.428 ± 0.16de
304 × 0µM	5.94 ± 0.66c	44 ± 7.91b	1.699 ± 0.16b
304 × 50µM	1.99 ± 0.66de	22 ± 7.91d	0.650 ± 0.16dc
304 × 100µM	0.59 ± 0.66ef	11 ± 7.91e	0.120 ± 0.16fe
0 × 0µM	0.01 ± 0.66f	5 ± 7.91e	0.005 ± 0.16f
0 × 50µM	0.01 ± 0.66f	5 ± 7.91e	0.005 ± 0.16f
0 × 100µM	0.01 ± 0.66f	5 ± 7.91e	0.005 ± 0.16f

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.3. Percent disease area, number of lesions and NDSU rating score associated with genotype × allopurinol interaction

Allopurinol × genotype interaction	Percent disease area	Number of lesions	NDSU rating score
0µM × HA89*5/HAR3	8.77 ± 0.76a	36 ± 8.06a	1.309 ± 0.17a
50µM × HA89*5/HAR3	4.02 ± 0.76cd	22 ± 8.06bc	0.551 ± 0.17bc
100µM × HA89*5/HAR3	1.14 ± 0.76fg	13 ± 8.06ed	0.145 ± 0.17cd
0µM × HA89*5/HAR2	9.18 ± 0.76a	36 ± 8.06a	1.250 ± 0.17a
50µM × HA89*5/HAR2	2.95 ± 0.76defg	19 ± 8.06bcd	0.296 ± 0.17bcd
100µM × HA89*5/HAR2	1.08 ± 0.76fg	9 ± 8.06e	0.109 ± 0.17d
0µM × HA89*5/cm90	5.20 ± 0.76bc	36 ± 8.06a	1.273 ± 0.17a
50µM × HA89*5/cm90	3.28 ± 0.76cde	24 ± 8.06b	0.670 ± 0.17b
100µM × HA89*5/cm90	0.99 ± 0.76g	12 ± 8.06de	0.199 ± 0.17cd
0µM × HA89	6.50 ± 076b	33 ± 8.06a	1.195 ± 0.17a
50µM × HA89	3.13 ± 0.76def	20 ± 8.06cd	0.452 ± 0.17bcd
100µM × HA89	1.25 ± 0.76efg	13 ± 8.06cde	0.270 ± 0.17bcd

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.4. Percent disease area, number of lesions and NDSU rating score associated with race × genotype × allopurinol interaction

Race × genotype × allopurinol interaction	Percent disease area	Number of lesions	NDSU rating score
336 × 0µM × HA89*5/HAR3	20.64 ± 1.29a	63 ± 9.10a	2.38 ± 0.27a
336 × 0µM × HA89*5/HAR2	20.56 ± 1.29a	58 ± 9.10ab	2.13 ± 0.27abc
336 × 0µM × HA89	14.78 ± 1.29b	56 ± 9.10abc	1.47 ± 0.27cdef
336 × 50µM × HA89*5/HAR3	9.61 ± 1.29c	35 ± 9.10defg	0.79 ± 0.27fghi
336 × 0µM × HA89*5/cm90	9.20 ± 1.29cd	45 ± 9.10bcd	2.29 ± 0.27ab
336 × 50µM × HA89*5/cm90	08.16 ± 1.29cde	45 ± 9.10bcd	1.28 ± 0.27defg
336 × 50µM × HA89*5/HAR2	7.78 ± 1.29cde	38 ± 9.10def	0.62 ± 0.27ghijk
304 × 0µM × HA89*5/HAR2	6.98 ± 1.29cde	43 ± 9.10cd	1.61 ± 0.27bcd
336 × 50µM × HA89	6.62 ± 1.29cdef	27 ± 9.10efgh	0.60 ± 0.27ghijk
304 × 0µM × HA89*5/cm90	6.34 ± 1.29cdefg	56 ± 9.10abc	1.53 ± 0.27cde
304 × 0µM × HA89*5/HAR3	5.67 ± 1.29defgh	40 ± 9.10de	1.55 ± 0.27cde
304 × 0µM × HA89	4.71 ± 1.29efghi	38 ± 9.10def	2.11 ± 0.27abc
336 × 100µM × HA89	3.20 ± 1.29fghij	24 ± 9.10fghi	0.73 ± 0.27ghij
336 × 100µM × HA89*5/HAR2	2.91 ± 1.29ghij	14 ± 9.10hijk	0.27 ± 0.27hijk
304 × 50µM × HA89	2.76 ± 1.29hij	27 ± 9.10efgh	0.75 ± 0.27ghij
336 × 100µM × HA89*5/cm90	2.53 ± 1.29hij	19 ± 9.10hijk	0.53 ± 0.27hijk
304 × 50µM × HA89*5/HAR3	2.46 ± 1.29hij	25 ± 9.10efghi	0.86 ± 0.27efgh
336 × 100µM × HA89*5/HAR3	2.33 ± 1.29hij	19 ± 9.10hijk	0.18 ± 0.27hijk
304 × 50 µM × HA89*5/cm90	1.66 ± 1.29ij	21 ± 9.10ghij	0.72 ± 0.27ghijk
304 × 100µM × HA89*5/HAR3	1.09 ± 1.29j	15 ± 9.10hijk	0.25 ± 0.27hijk
304 × 50µM × HA89*5/HAR2	1.06 ± 1.29j	14 ± 9.10hijk	0.26 ± 0.27hijk
304 × 100µM × HA89	0.55 ± 1.29j	11 ± 9.10ijk	0.08 ± 0.27ijk
304 × 100µM × HA89*5/cm90	0.43 ± 1.29j	11 ± 9.10ijk	0.06 ± 0.27jk
304 × 100µM × HA89*5/HAR2	0.31 ± 1.29j	9 ± 9.10jk	0.05 ± 0.27jk
0 × 0 µM × HA89*5/HAR2	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k
0 × 50µM × HA89*5/HAR2	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k
0 × 50µM × HA89*5/HAR3	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k
0 × 100µM × HA89	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k
0 × 100µM × HA89*5/cm90	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k
0 × 0µM × HA89*5/HAR3	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k
0 × 0µM × HA89	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k
0 × 50µM × HA89	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k
0 × 100µM × HA89*5/HAR2	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k
0 × 100µM × HA89*5/HAR3	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k
0 × 50µM × HA89*5/cm90	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k
0 × 0µM × HA89*5/cm90	0.01 ± 1.29j	5 ± 9.10k	0.00 ± 0.27k

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.5. Days to maturity, plant height and weight of stalks associated with race × genotype interactions

Race × genotype interaction	Days to maturity	Plant height (cm)	Weight of stalks (g)
336 × HA89*5/HAR3	120 ± 1.5de	128.48 ± 13.09ab	37.28 ± 16.29a
336 × HA89*5/HAR2	122 ± 1.5bcd	122.09 ± 13.09def	37.17 ± 16.29a
336 × HA89	121 ± 1.5cde	121.36 ± 13.09ef	36.98 ± 16.29a
336 × HA89*5/cm90	119 ± 1.5e	126.05 ± 13.09bcde	37.42 ± 16.29a
304 × HA89*5/HAR3	124 ± 1.5abc	127.42 ± 13.09abcd	31.17 ± 16.29e
304 × HA89*5/cm90	124 ± 1.5ab	124.73 ± 13.09bcde	31.83 ± 16.29de
304 × HA89*5/HAR2	122 ± 1.5bcd	118.53 ± 13.09f	32.28 ± 16.29cde
304 × HA89	126 ± 1.5a	122.89 ± 13.09cdef	32.55 ± 16.29bcde
0 × HA89*5/HAR2	124 ± 1.5ab	118.16 ± 13.09f	32.17 ± 16.29de
0 × HA89	123 ± 1.5bcd	125.39 ± 13.09bcde	33.78 ± 16.29bcd
0 × HA89*5/HAR3	123 ± 1.5bcd	131.95 ± 13.09a	34.75 ± 16.29b
0 × HA89*5/cm90	122 ± 1.5bcd	128.03 ± 13.09abc	34.43 ± 16.29bc

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.6. Days to maturity, plant height and weight of stalks associated with race × allopurinol interactions

Race × allopurinol interaction	Days to maturity	Plant height (cm)	Weight of talks (g)
336 × 0µM	122 ± 1.4bc	123.57 ± 13.06ab	36.65 ± 16.29b
336 × 50µM	120 ± 1.4cd	124.71 ± 13.06a	38.63 ± 16.29a
336 × 100µM	120 ± 1.4d	125.20 ± 13.06a	36.36 ± 16.29b
304 × 0µM	123 ± 1.4ab	125.76 ± 13.06a	31.89 ± 16.29c
304 × 50µM	124 ± 1.4a	124.68 ± 13.06a	32.49 ± 16.29c
304 × 100µM	125 ± 1.4a	119.73 ± 13.06b	31.49 ± 16.29c
0 × 0µM	124 ± 1.4ab	127.39 ± 13.06a	35.93 ± 16.29b
0 × 50µM	122 ± 1.4bcd	123.92 ± 13.06ab	32.53 ± 16.29c
0 × 100µM	124 ± 1.4ab	126.35 ± 13.06a	32.89 ± 16.29c

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.7. Days to maturity, plant height and weight of stalks associated with allopurinol × genotype interactions

Allopurinol × genotype interaction	Days to maturity	Plant height (cm)	Weight of stalks (g)
0µM × HA89*5/HAR3	121± 1.45ab	132.38 ± 13.09a	34.53 ± 16.29abcd
50µM × HA89*5/HAR3	122 ± 1.45ab	128.98 ± 13.09ab	36.20 ± 16.29a
100µM × HA89*5/HAR3	124 ± 1.45ab	126.49 ± 13.09bc	32.47 ± 16.29d
0µM × HA89*5/HAR2	124 ± 1.45ab	120.52 ± 13.09de	34.67 ± 16.29abcd
50µM × HA89*5/HAR2	122 ± 1.45ab	119.36 ± 13.09e	33.92 ± 16.29bcd
100µM × HA89*5/HAR2	123 ± 1.45ab	118.90 ± 13.09e	32.58 ± 16.29cd
0µM × HA89*5/cm90	122 ± 1.45ab	127.23 ± 13.09abc	34.78 ± 16.29abc
50µM × HA89*5/cm90	122 ± 1.45ab	126.11 ± 13.09bc	34.64 ± 16.29abcd
100µM × HA89*5/cm90	121 ± 1.45b	125.47 ± 13.09bcd	34.71 ± 16.29abc
0µM × HA89	124 ± 1.45ab	122.16 ± 13.09cde	35.32 ± 16.29ab
50µM × HA89	122 ± 1.45ab	123.30 ± 13.09cde	33.45 ± 16.29bcd
100µM×HA89	124 ± 1.45a	124.17 ± 13.09bcde	34.55 ± 16.29abcd

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.8. Days to maturity, plant height and weight of stalks associated with race × genotype × allopurinol interaction

Race × genotype × allopurinol interaction	Days to maturity	Plant height (cm)	Weight of stalks (g)
336 × 0µM × HA89*5/HAR3	119 ± 2ghi	125.66 ± 13.4bcdefghi	34.85 ± 16.3defghij
336 × 0µM × HA89*5/HAR2	124 ± 2abcdef	120.75 ± 13.4defghij	37.73 ± 16.3bcdef
336 × 0µM × HA89	123 ±2bcdefgh	120.79 ± 13.4defghij	37.19 ± 16.3bcdefg
336 × 50µM × HA89*5/HAR3	121 ± 2defgh	120.35 ± 13.4defg	42.04 ± 16.3a
336 × 0µM × HA89*5/cm90	123 ± 2bcdefg	127.08 ± 13.4bcdefgh	36.82 ± 16.3bcdefg
336 × 50µM × HA89*5/cm90	118 ± 2hi	123.59 ± 13.4cdefghij	38.44 ± 16.3abcd
336 × 50µM × HA89*5/HAR2	121 ± 2defgh	123.12 ± 13.4cdefghij	39.00 ± 16.3ab
304 × 0µM × HA89*5/HAR2	124 ± 2abcdef	120.41 ± 13.4defghij	30.22 ± 16.3lmn
336 × 50µM × HA89	120 ± 2efgh	123.60 ± 13.4cdefghij	35.05 ± 16.3cdefghij
304 × 0µM × HA89*5/cm90	121 ± 2defgh	129.65 ± 13.4abcde	33.79 ± 16.3hijkl
304 × 0µM × HA89*5/HAR3	122 ± 2cdefgh	133.92± 13.4ab	32.60 ± 16.3ijklm
304 × 0µM × HA89	125 ± 2abcdef	119.07 ± 13.4fghij	30.94 ± 16.3klmn
336 × 100µM × HA89	121 ± 2defgh	119.68 ± 13.4efghij	38.69 ± 16.3abc
336 × 100µM × HA89*5/HAR2	121 ± 2defgh	122.40 ± 13.4cdefghij	34.78 ± 16.3defghij
304 × 50µM × HA89	127 ± 2ab	126.98 ± 13.4bcdefgh	33.78 ± 16.3ghijkl
336 × 100µM × HA89*5/cm90	115 ± 2i	127.48 ± 13.4abcdefg	36.99 ± 16.3bcdefg
304 × 50µM × HA89*5/HAR3	125 ± 2abcdef	128.58 ± 13.4abcde	32.23 ± 16.3jklmn
336 × 100µM × HA89*5/HAR3	122 ± 2cdefgh	131.24 ± 13.4abc	34.96 ± 16.3cdefghij
304 × 50 µM × HA89*5/cm90	126 ± 2abc	126.23 ± 13.4bcdefghi	32.63 ± 16.3ijklm
304 × 100µM × HA89*5/HAR3	125 ± 2abcd	119.75 ± 13.4efghij	28.66 ± 16.3n
304 × 50µM × HA89*5/HAR2	120 ± 2fgh	116.92 ± 13.4ij	31.33 ± 16.3jklmn
304 × 100µM × HA89	128 ± 2a	122.61 ± 13.4cdefghij	32.93 ± 16.3hijkl
304 × 100µM × HA89*5/cm90	125 ± 2abcd	118.30 ± 13.4ghij	30.42 ± 16.3lmn
304 × 100µM × HA89*5/HAR2	122 ± 2cdefgh	118.24 ± 13.4ghij	33.93 ± 16.3fghijkl
0 × 0 µM × HA89*5/HAR2	124 ± 2abcdef	120.40 ± 13.4defghij	36.06 ± 16.3bcdefgh
0 × 50µM × HA89*5/HAR2	125 ± 2abcdef	118.03 ± 13.4hij	31.41 ± 16.3jklmn
0 × 50µM × HA89*5/HAR3	121 ± 2cdefgh	129.82 ± 13.4abcd	34.32 ± 16.3efghijk
0 × 100µM × HA89	124 ± 2abcdef	130.22 ± 13.4abc	32.02 ± 16.3jklmn
0 × 100µM × HA89*5/cm90	123 ± 2bcdefg	130.63 ± 13.4abc	36.72 ± 16.3bcdefgh
0 x 0µM x HA89*5/HAR3	124 ± 2abcdef	135.56 ± 13.4a	36.13 ± 16.3bcdefgh
0 x 0µM x HA89	125 ± 2abcdef	126.62 ± 13.4bcdefgh	37.81 ± 16.3bcde
0 x 50µM x HA89	120 ± 2efgh	119.32 ± 13.4efghij	31.51 ± 16.3jklmn
0 x 100µM x HA89*5/HAR2	125 ± 2abcdef	116.06 ± 13.4j	29.02 ± 16.3nm
0 x 100µM x HA89*5/HAR3	124 ± 2abcdef	128.48 ± 13.4abcdef	33.78 ± 16.3ghijkl
0 x 50µM x HA89*5/cm90	121 ± 2defgh	128.51 ± 13.4abcde	32.86 ± 16.3jikl
0 x 0µM x HA89*5/cm90	122 ± 2bcdefg	124.96 ± 13.4bcdefghi	33.72 ± 16.3ghijkl

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.9. Area of head, weight of head and number of seeds associated with race × genotype interactions

Race × genotype interaction	Area of head (cm^2)	Weight of head (g)	Number of seeds
336 × HA89*5/HAR3	58.41 ± 24.9ab	26.94 ± 10.5a	338 ± 187.4ab
336 × HA89*5/HAR2	60.33 ± 24.9a	25.46 ± 10.5abcd	327 ± 187.4abc
336 × HA89	57.79 ± 24.9abc	24.76 ± 10.5abcd	306 ± 187.4bcd
336 × HA89*5/cm90	54.73 ± 24.9bcd	24.56 ± 10.5abcd	355 ± 187.4a
304 × HA89*5/HAR3	51.43 ± 24.9def	24.13 ± 10.5bcd	314 ± 187.4bcd
304 × HA89*5/cm90	47.07 ± 24.9f	23.92 ± 10.5cd	264 ± 187.4e
304 × HA89*5/HAR2	49.27 ± 24.9ef	23.14 ± 10.5d	222 ± 187.4f
304 × HA89	52.93 ± 24.9cde	26.29 ± 10.5abc	255 ± 187.4ef
0 × HA89*5/HAR2	56.34 ± 24.9abcd	26.61 ± 10.5ab	219 ± 187.4de
0 × HA89	56.06 ± 24.9abcd	25.88 ± 10.5abc	299 ± 187.4cd
0 × HA89*5/HAR3	54.96 ± 24.9bcd	24.22 ± 10.5bcd	339 ± 187.4ab
0 × HA89*5/cm90	56.48 ± 24.9abcd	25.29 ± 10.5abcd	317 ± 187.4bc

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.10. Area of head, weight of head and number of seeds associated with race × allopurinol interactions

Race × allopurinol interaction	Area of head (cm^2)	Weight of heads (g)	Number of seeds
336 × 0µM	60.34 ± 24.9a	25.84 ± 10.5ab	342 ± 187.3b
336 × 50µM	60.22 ± 24.9ab	25.89 ± 10.5ab	377 ± 187.3a
336 × 100µM	52.88 ± 24.9cd	24.57 ± 10.5bc	275 ± 187.3c
304 × 0µM	51.87 ± 24.9cd	24.47 ± 10.5bc	278 ± 187.3c
304 × 50µM	49.99 ± 24.9d	22.73 ± 10.5c	263 ± 187.3c
304 × 100µM	48.67 ± 24.9d	25.91 ± 10.5ab	252 ± 187.3c
0 × 0µM	59.54 ± 24.9ab	26.92 ± 10.5a	332 ± 187.3b
0 × 50µM	52.54 ± 24.9cd	24.33 ± 10.5bc	276 ± 187.3c
0 × 100µM	55.80 ± 24.9bc	25.24 ± 10.5ab	320 ± 187.3b

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.11. Area of head, weight of head and number of seeds associated with allopurinol × genotype interactions

Allopurinol × genotype interaction	Area of head (cm^2)	Weight of heads (g)	Number of seeds
0µM × HA89*5/HAR3	57.49 ± 24.9ab	25.19 ± 10.5ab	347 ± 187.4a
50µM × HA89*5/HAR3	56.17 ± 24.9abc	24.29 ± 10.5ab	322 ± 187.4ab
100µM × HA89*5/HAR3	51.14 ± 24.9c	25.83 ± 105ab	322 ± 187.4ab
0µM × HA89*5/HAR2	57.81 ± 24.9ab	26.80 ± 10.5a	277 ± 187.4cd
50µM × HA89*5/HAR2	53.95 ± 24.9bc	24.99 ± 10.5	319 ± 187.4ab
100µM × HA89*5/HAR2	54.17 ± 24.9bc	23.43 ± 10.5b	237 ± 187.4e
0µM × HA89*5/cm90	52.75 ± 24.9bc	24.24 ± 10.5ab	330 ± 187.4ab
50µM × HA89*5/cm90	53.79 ± 24.9bc	24.35 ± 10.5ab	307 ± 187.4bc
100µM x HA89*5/cm90	51.74 ± 24.9c	25.18 ± 10.5ab	299 ± 187.4bcd
0µM × HA89	60.95 ± 24.9a	26.76 ± 10.5a	315 ± 187.4ab
50µM × HA89	53.09 ± 24.9bc	23.65 ± 10.5b	273 ± 187.4cd
100µM × HA89	52.75 ± 24.9bc	25.52 ± 10.5a	271 ± 187.4d

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.12. Area of head, weight of head and number of seeds with race × genotype × allopurinol interaction

Race × genotype × allopurinol interaction	Area of head (cm^2)	Weight of head (g)	Number of seeds
336 × 0µM × HA89*5/HAR3	59.91 ± 25.1abcde	25.73 ± 10.6defgh	345 ± 188bcdef
336 × 0µM × HA89*5/HAR2	63.45 ± 25.1ab	27.76 ± 10.6bcde	286 ± 188fghijklm
336 × 0µM × HA89	68.07 ± 25.1a	28.53 ± 10.6abcd	372 ± 188bc
336 × 50µM × HA89*5/HAR3	62.58 ± 25.1abc	24.89 ± 10.6defghi	351 ± 188bcd
336 × 0µM × HA89*5/cm90	49.92 ± 25.1fghi	21.34 ± 10.6hi	363 ± 188bcd
336 × 50µM × HA89*5/cm90	58.54 ± 25.1bcdef	27.16 ± 10.6bcdef	371 ± 188bc
336 × 50µM × HA89*5/HAR2	62.50 ± 25.1abc	27.28 ± 10.6bcdef	466 ± 188a
304 × 0µM × HA89*5/HAR2	47.89 ± 25.1ghij	21.88 ± 10.6ghi	207 ± 188p
336 × 50µM × HA89	57.27± 25.1bcdef	24.23 ± 10.6defghi	320 ± 188cdefghi
304 × 0µM × HA89*5/cm90	51.11 ± 25.1efghi	25.99 ± 10.6cdefg	285 ± 188ghjiklmn
304 × 0µM × HA89*5/HAR3	55.62 ± 25.1bcdefg	25.99 ± 10.6cdefg	349 ± 188bcde
304 × 0µM × HA89	52.87 ± 25.1efghi	24.04 ± 10.6efghi	269 ± 188ljklmno
336 × 100µM × HA89	48.04 ± 25.1ghij	21.52 ± 10.6hi	225 ± 188nop
336 × 100µM × HA89*5/HAR2	55.03 ± 25.1bcdefgh	21.34 ± 10.6hi	228 ± 188mnop
304 × 50µM × HA89	50.20 ± 25.1fghi	22.45 ± 10.6ghi	242 ± 188lmnop
336 × 100µM × HA89*5/cm90	55.73 ± 25.1bcdefg	25.20 ± 10.6defghi	331 ± 188bcdefgh
304 × 50µM × HA89*5/HAR3	52.66 ± 25.1efghi	23.44 ± 10.6efghi	326 ± 188bcdefghi
336 × 100µM × HA89*5/HAR3	52.73 ± 25.1efghi	30.21 ± 10.6abc	317 ± 188cdefghij
304 × 50 µM × HA89*5/cm90	50.47 ± 25.1fghi	21.08 ± 10.6i	268 ± 188ijklmno
304 × 100µM × HA89*5/HAR3	46.02 ± 25.1ij	22.96 ± 10.6fghi	269 ± 188ijklmno
304 × 50µM × HA89*5/HAR2	46.61 ± 25.1hij	23.94 ± 10.6efghi	214 ± 188op
304 × 100µM × HA89	55.72 ± 25.1bcdefg	32.37 ± 10.6a	253 ± 188klmnop
304 × 100µM × HA89*5/cm90	39.64 ± 25.1j	24.69 ± 10.6defghi	240 ± 188lmnop
304 × 100µM × HA89*5/HAR2	53.32 ± 25.1defghi	23.61 ± 10.6efghi	246 ± 188klmnop
0 × 0 µM × HA89*5/HAR2	62.10 ± 25.1abcd	30.75 ± 10.6ab	337 ± 188bcdefg
0 × 50µM × HA89*5/HAR2	52.74 ± 25.1efghi	23.74 ± 10.6efghi	275 ± 188hijklmn
0 × 50µM × HA89*5/HAR3	53.27 ± 25.1defghi	24.53 ± 10.6defghi	290 ± 188efghijkl
0 × 100µM × HA89	54.48 ± 25.1cdefghi	25.67 ± 10.6defgh	337 ± 188bcdefg
0 × 100µM × HA89*5/cm90	59.88 ± 25.1abcde	25.64 ± 10.6defgh	328 ± 188bcdefghi
0 × 0µM × HA89*5/HAR3	56.96 ± 25.1bcdef	23.84 ± 10.6efghi	346 ± 188bcde
0 × 0µM × HA89	61.91 ± 25.3abcd	27.70 ± 10.6bcde	304 ± 188defghijk
0 × 50µM × HA89	51.79 ± 25.1efghi	24.27 ± 10.6defghi	257 ± 188jklmnop
0 × 100µM × HA89*5/HAR2	54.16 ± 25.1cdefghi	25.34 ± 10.6defghi	234 ± 188lmnop
0 × 100µM × HA89*5/HAR3	54.67 ± 25.1bcdefghi	24.31 ± 10.6defghi	381 ± 188b
0 × 50µM × HA89*5/cm90	52.36 ± 25.1efghi	24.79 ± 10.6defghi	283 ± 188ghijklmn
0 × 0µM × HA89*5/cm90	57.19 ± 25.1bcdef	25.40 ± 10.6defghi	341 ± 188bcdefg

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.13. Weight of seeds, weight of biomass and harvest index associated with race × genotype interactions

Race × genotype interaction	Weight of seeds (g)	Weight of biomass (g)	Harvest index
336 × HA89*5/HAR3	12.71 ± 7.5a	65.66 ± 26.8a	0.168 ± 0.049bcd
336 × HA89*5/HAR2	12.02 ± 7.5ab	61.74 ± 26.8bc	0.183 ± 0.049ab
336 × HA89	12.38 ± 7.5a	61.79 ± 26.8bc	0.185 ± 0.049ab
336 × HA89*5/cm90	12.32 ± 7.5ab	62.32 ± 26.8ab	0.176 ± 0.049abc
304 × HA89*5/HAR3	11.99 ± 7.5ab	55.79 ± 26.8ef	0.184 ± 0.049ab
304 × HA89*5/cm90	10.32 ± 7.5cd	55.93 ± 26.8ef	0.158 ± 0.049cd
304 × HA89*5/HAR2	09.40 ± 7.5d	54.68 ± 26.8f	0.151 ± 0.049d
304 × HA89	11.16 ± 7.5bc	57.78 ± 26.8def	0.175 ± 0.049abc
0 × HA89*5/HAR2	11.96 ± 7.5ab	58.35 ± 26.8cdef	0.174 ± 0.049bcd
0 × HA89	12.11 ± 7.5ab	59.87 ± 26.8bcd	0.190 ± 0.049ab
0 × HA89*5/HAR3	12.70 ± 7.5a	59.23 ± 26.8bcde	0.188 ± 0.049ab
0 × HA89*5/cm90	12.87 ± 7.5a	59.89 ± 26.8bcd	0.198 ± 0.049a

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.14. Weight of seeds, weight of biomass and harvest index associated with race × allopurinol interactions

Race × allopurinol interaction	Weight of seeds (g)	Weight of biomass (g)	Harvest index
336 × 0µM	13.30 ± 7.5ab	62.42 ± 26.8ab	0.194 ± 0.049a
336 × 50µM	13.45 ± 7.5ab	64.78 ± 26.8a	0.183 ± 0.049ab
336 × 100µM	10.31 ± 7.5c	61.43 ± 26.8b	0.157 ± 0.049c
304 × 0µM	10.95 ± 7.5c	56.43 ± 26.8c	0.165 ± 0.049bc
304 × 50µM	10.62 ± 7.5c	55.60 ± 26.8c	0.166 ± 0.049bc
304 × 100µM	10.57 ± 7.5c	56.11 ± 26.8c	0.169 ± 0.049bc
0 × 0µM	13.75 ± 7.5a	63.67 ± 26.8ab	0.191 ± 0.049a
0 × 50µM	10.92 ± 7.5c	56.71 ± 26.8c	0.176 ± 0.049abc
0 × 100µM	12.56 ± 7.5b	57.62 ± 26.8c	0.195 ± 0.049a

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.15. Weight of seeds, weight of biomass and harvest index associated with allopurinol × genotype interactions

Allopurinol × genotype interaction	Weight of seeds (g)	Weight of biomass (g)	Harvest index
0µM × HA89*5/HAR3	13.31 ± 7.5ab	60.75 ± 26.8abc	0.189 ± 0.049a
50µM × HA89*5/HAR3	12.05 ± 7.5c	61.60 ± 26.8ab	0.159 ± 0.049bc
100µM × HA89*5/HAR3	12.04 ± 7.5c	58.33 ± 26.8bcd	0.191 ± 0.049a
0µM × HA89*5/HAR2	12.09 ± 7.5bc	60.61 ± 26.8abc	0.177 ± 0.049ab
50µM × HA89*5/HAR2	11.66 ± 7.5c	58.36 ± 26.8bcd	0.181 ± 0.049ab
100µM × HA89*5/HAR2	09.62 ± 7.5d	55.81 ± 26.8d	0.149 ± 0.049c
0µM × HA89*5/cm90	11.91 ± 7.5c	59.43 ± 26.8abcd	0.174 ± 0.049ab
50µM × HA89*5/cm90	11.95 ± 7.5c	58.91 ± 26.8abcd	0.188 ± 0.049a
100µM × HA89*5/cm90	11.64 ± 7.5c	59.80 ± 26.8abc	0.170 ± 0.049abc
0µM × HA89	13.36 ± 7.5a	62.58 ± 26.8a	0.194 ± 0.049a
50µM × HA89	10.99 ± 7.5c	57.24 ± 26.8cd	0.173 ± 0.049abc
100µM × HA89	11.29 ± 7.5c	59.61 ± 26.8abcd	0.184 ± 0.049a

Least square means followed by same letter are not different. Numbers followed by ± are the standard error

Appendix 3.16. Weight of seeds, weight of biomass and harvest index with race × genotype × allopurinol interaction

Race × genotype × allopurinol interaction	Weight of seed (g)	Weight of biomass (g)	Harvest Index
336 × 0µM × HA89*5/HAR3	13.19 ± 7.51bcdef	60.98 ± 26.89bcdefghi	0.193 ± 0.050abcde
336 × 0µM × HA89*5/HAR2	12.82 ± 7.51bcdef	63.69 ± 26.89abcdef	0.191 ± 0.050bcde
336 × 0µM × HA89	15.74 ± 7.51a	65.97 ± 26.89abc	0.221 ± 0.050ab
336 × 50µM × HA89*5/HAR3	13.45 ± 7.51bcdef	69.16 ± 26.89a	0.145 ± 0.050hi
336 × 0µM × HA89*5/cm90	11.45 ± 7.51fghijk	59.04 ± 26.89defghijkl	0.170 ± 0.050efghi
336 × 50µM × HA89*5/cm90	14.02 ± 7.51abc	65.59 ± 26.89abcd	0.198 ± 0.050abcde
336 × 50µM × HA89*5/HAR2	13.79 ± 7.51abcd	65.13 ± 26.89abcde	0.195 ± 0.050abcde
304 × 0µM × HA89*5/HAR2	08.92 ± 7.51l	50.94 ± 26.89m	0.149 ± 0.050fghi
336 × 50µM × HA89	12.54 ± 7.51bcdefg	59.23 ± 26.89defghijkl	0.194 ± 0.050abcde
304 × 0µM × HA89*5/cm90	10.49 ± 7.51ghijkl	59.62 ± 26.89cdefghijkl	0.139 ± 0.050i
304 × 0µM × HA89*5/HAR3	13.04 ± 7.51bcdef	59.66 ± 26.89cdefghijkl	0.185 ± 0.050bcdefgh
304 × 0µM × HA89	11.36 ± 7.51fghijk	55.50 ± 26.89hijklm	0.187 ± 0.050bcdefg
336 × 100µM × HA89	08.84 ± 7.51l	60.15 ± 26.89cdefghijk	0.140 ± 0.050i
336 × 100µM × HA89*5/HAR2	09.44 ± 7.51kl	56.40 ± 26.89ghijklm	0.162 ± 0.050efghi
304 × 50µM × HA89	10.54 ± 7.51ghijkl	56.50 ± 26.89ghijklm	0.161 ± 0.050efghi
336 × 100µM × HA89*5/cm90	11.48 ± 7.51fghijk	62.35 ± 26.89bcdefg	0.160 ± 0.050efghi
304 × 50µM × HA89*5/HAR3	12.26 ± 7.51cdefgh	56.83 ± 26.89ghijklm	0.174 ± 0.050defghi
336 × 100µM × HA89*5/HAR3	11.49 ± 7.51fghijk	66.83 ± 26.89ab	0.167 ± 0.050efghi
304 × 50 µM × HA89*5/cm90	10.12 ± 7.51ijkl	53.76 ± 26.89klm	0.167 ± 0.050efghi
304 × 100µM × HA89*5/HAR3	10.68 ± 7.51ghijkl	50.89 ± 26.89m	0.191 ± 0.050bcde
304 × 50µM × HA89*5/HAR2	09.57 ± 7.51jkl	55.30 ± 26.89hijklm	0.164 ± 0.050efghi
304 × 100µM × HA89	11.58 ± 7.51fghij	61.34 ± 26.89bcdefghi	0.178 ± 0.050cdefghi
304 × 100µM × HA89*5/cm90	10.34 ± 7.51hijkl	54.40 ± 26.89jklm	0.167 ± 0.050efghi
304 × 100µM × HA89*5/HAR2	09.71 ± 7.51ijkl	57.81 ± 26.89fghijkl	0.141 ± 0.050i
0 × 0 µM × HA89*5/HAR2	14.54 ± 7.51ab	67.18 ± 26.89ab	0.192 ± 0.050bcde
0 × 50µM × HA89*5/HAR2	11.63 ± 7.51efghi	54.67 ± 26.89ijklm	0.184 ± 0.050bcdefgh
0 × 50µM × HA89*5/HAR3	10.45 ± 7.51ghijkl	58.81 ± 26.89efghijkl	0.160 ± 0.050efghi
0 × 100µM × HA89	13.45 ± 7.51bcdef	57.36 ± 26.89fghijklm	0.234 ± 0.050a
0 × 100µM × HA89*5/cm90	13.10 ± 7.51bcdef	62.66 ± 26.89abcdefg	0.183 ± 0.050bcdefgh
0 × 0µM × HA89*5/HAR3	13.70 ± 7.51abcde	61.61 ± 26.89bcdefgh	0.187 ± 0.050bcdef
0 × 0µM × HA89	12.99 ± 7.51bcdef	66.27 ± 26.89abc	0.174 ± 0.050defghi
0 × 50µM × HA89	09.89 ± 7.51ijkl	55.99 ± 26.89ghijlkm	0.164 ± 0.050efghi
0 × 100µM × HA89*5/HAR2	09.71 ± 7.51ijkl	53.20 ± 26.89lm	0.146 ± 0.050ghi
0 × 100µM × HA89*5/HAR3	13.95 ± 7.51abc	57.27 ± 26.89fghijklm	0.216 ± 0.050abc
0 × 50µM × HA89*5/cm90	11.71 ± 7.51defghi	57.37 ± 26.89fghijklm	0.198 ± 0.050abcde
0 × 0µM × HA89*5/cm90	13.78 ± 13.78abcd	59.64 ± 26.89cdefghijkl	0.212 ± 0.050abcd

Least square means followed by same letter are not different. Numbers followed by ± are the standard error